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THE LONGEVITY OF THE TYPHOID BACILLUS IN WATER.*

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INTRODUCTION.

THE writers of this paper were requested by the Sanitary District of Chicago, in the fall of 1903, to conduct some experiments upon the life of the typhoid bacillus in the waters of Lake Michigan, the Chicago Drainage Canal, and the Illinois River. The purpose of these experiments was to shed light upon the question as to whether the typhoid bacillus could survive the passage from the Chicago Drainage Canal to the mouth of the Illinois River, under the conditions obtaining in the latter stream. The particular circumstances leading to this inquiry have been fully set forth by one of us elsewhere¹ and need not be here rehearsed.

Previous experiments that have been made to determine the longevity of the typhoid bacillus in water have been conducted, for the most part, if not wholly, in glass vessels in a laboratory, and have been carried on under such varied conditions of temperature, character of water, and number and age of bacteria introduced, that widely divergent conclusions have been reached by different observers. From these experiments, however, substantial accord has resulted on two points: first, that typhoid bacilli die out more

* Received for publication May 10, 1904.

¹ JORDAN, *Jour. Exper. Med.*, 1901, 5, p. 271.

rapidly in unsterilized water than in the same water sterilized by heat; second, that when typhoid bacilli are introduced into unsterilized water containing little organic matter, their longevity is more prolonged than in water charged with considerable organic matter.¹ As regards application of results hitherto obtained to particular cases the available evidence appears to indicate that the conditions of laboratory experiments are so far removed from the conditions found in nature as to afford conclusions of doubtful value. Especially in experimental work designed to test the so-called self-purification of streams it would seem desirable to conform to natural conditions, so far as practicable, in order to secure any very cogent evidence.

Division of the work.—The work at the several stations selected for observation was definitely assigned to individuals who were responsible for the carrying out of details. In this way practically three independent experiments were executed simultaneously with the same strains of typhoid cultures. The details of the work were varied somewhat, yet the methods pursued were essentially the same in all cases.

The proximity of four of the experiment stations to Chicago rendered it possible to conduct the work in the laboratories of the Northwestern University and the University of Chicago. Acknowledgment is made in this connection of the services of those who have been engaged in various parts of the investigation.

Experiments upon Lake Michigan and Chicago River water were carried out by Professor F. R. Zeit, with the co-operation of Dr. V. H. Bassett. Dr. Bassett also added some independent experiments on the longevity of the dysentery bacillus, which are incorporated in this paper.

Experiments on the Drainage Canal at its upper (Robey Street) and lower (Lockport) ends were conducted by Professor E. O. Jordan, with the assistance of Dr. Norman MacLeod Harris, Dr. Ernest E. Irons, and Miss Mary C. Lincoln. We are especially indebted to Dr. Harris for the preparation and testing of the special culture media used in the work of isolation. A special report by him on the comparative value of the media will follow in a later number of this JOURNAL.

The studies on the Illinois River, under the direction of Professor H. L. Russell, were carried out in the biological laboratory of the Bradley Polytechnic Institute at Peoria, Ill., the facilities of which were kindly placed at our disposal by Professor Wales M. Packard. The work of securing the samples and the isolation of presumptive typhoid colonies was done by Mr. E. G. Hastings. The cultures were then sent to the University of Wisconsin at Madison, where they were subjected to further study and identification by Professor Russell, who was assisted in this work by Professor W. D. Frost and G. J. Marquette.

¹ *E. g.*, FRANKLAND, *Ztschr. f. Hyg.*, 1895, p. 19, 333.

TECHNIQUE.

Isolation of cultures.—It was considered important to employ freshly isolated typhoid cultures in the experiments, since one of the writers¹ had shown that a difference exists, as regards longevity in water, between freshly isolated strains and those kept under cultivation for some time. Three cultures, designated respectively as *B. typhosus* “x,” “y,” and “z,” were accordingly procured for the purpose.

B. typhosus x was isolated on September 22, 1903, from the blood of a typhoid fever patient. The symptomatology of this case was typical, and the bacillus was obtained in pure culture from the blood. The case terminated fatally, and the autopsy showed the characteristic lesions.

B. typhosus y was isolated October 1, 1903, from the urine of a typhoid fever patient, showing typical symptoms, and responding positively on the tenth day of the disease to the agglutination test. This bacillus was used in pure culture for some inoculations, and the urine of this patient, containing typhoid bacilli, was also used for direct inoculation into some of the sacs.

B. typhosus z was isolated September 28, 1903, from the feces of a typhoid fever patient. The case was perfectly typical and terminated fatally.

These three strains were critically examined as regards all of their biological characters, and proved to be thoroughly typical cultures of the typhoid bacillus. All three agglutinated in high dilutions (1:1,000) with the serum of a rabbit immunized with the University of Chicago laboratory stock culture of *B. typhosus*.²

The use of these newly isolated strains, and their introduction, both directly and in the urine of the typhoid fever patient, into the waters studied, was thought to afford a reasonably close approximation to natural conditions.

Method of exposing bacilli to canal and river waters.—The organisms in a flowing stream are subjected to a more or less changing environment, varying according to local circumstances; hence, if natural conditions were to be precisely imitated, it would be necessary to subject the bacteria to these changing conditions. Manifestly such a course as this would be difficult to carry into effect, but the difficulty may be partly overcome by selecting for experi-

¹ JORDAN, *Med. News*, Sept. 28, 1895.

² These freshly isolated cultures did not, however, agglutinate in as high dilutions as typhoid strains that had been under cultivation in this laboratory for some months or years. These latter agglutinated in dilutions of about 1:10,000 with this same serum. — E. O. J.

mentation different points along a stream, choosing these so as to represent wide ranges in conditions. In the present case a great difference in conditions exists between the highly polluted Chicago River or the upper portion of the Drainage Canal, and the purified water in the lower stretches of the Illinois River. Accordingly the following stations were selected for experiment:

TABLE I.

No.	Station	Distance from Lake Michig'n	Time of Flow
		Miles	Days
1.....	Lake Michigan (tap supply at 2400 Dearborn St. derived from "Four-Mile Crib").....	0	0
2.....	Chicago River, Ashland Ave.....	5½	..
3.....	Head of Drainage Canal, Robey St., Chicago....	6	..
4.....	Mouth of Drainage Canal, Bear Trap Dam, Lockport, Ill.....	34	2
5.....	Illinois River, Averyville.....	125	5-8

By exposing the several strains of typhoid organisms to these various waters it was thought that the range in conditions would be nearly as great as would be encountered in the natural passage downstream.

Use of permeable sacs.—The errors introduced by using ordinary glass containers in work of this character have been clearly shown by Ficker,¹ and again very recently by Hesse.² In our experiments the attempt has been made to expose the typhoid organism under as nearly natural conditions as possible, taking into consideration the difficulties which necessarily obtain in recovering the organism from the water. For this purpose permeable sacs of parchment or celloidin were employed, which were filled with canal or river water taken at the respective stations, and then infected with typhoid bacilli.

Celloidin sacs.—Celloidin capsules were first used in bacteriological work by Mörpurgo and Tirelli in 1892³ for the cultivation of tubercle bacilli. The method has since been used and perfected by various investigators with more or less success. Metchnikoff, Roux, and Salimbeni⁴ used it for their studies of cholera toxin; Nocard and Roux,⁵ for their studies of pleuropneumonia in cattle; and Nocard,⁶ in his attempt to transform human into avian tubercle bacilli. McCrae⁷ in his studies of agglutination devised a simpler

¹ *Ztschr. f. Hyg.*, 1895, 29, p. 1.² *Ibid.*, 1904, 46, p. 1.³ *Archives ital. de Biol.*, 1892, 18, p. 187.⁴ *Ann. de l'Inst. Past.*, 1896, 10, p. 257.⁵ *Ibid.*, 1898, 12, p. 270.⁶ *Ibid.*, 1898, 12, p. 561.⁷ *Jour. Exper. Med.*, 1901, 5, p. 635.

and more practical method, which was still further perfected by Harris.¹

The successful use of celloidin sacs in bacteriology by these investigators led one of us (F. R. Z.) to employ Harris's method in these experiments.

The sac became somewhat brittle and less flexible after about ten days' exposure in the water. Breaks occurred but rarely, and then only at the joint of the glass tube and sac, or along the middle of the sac, where the original joint of the gelatin capsule had been. In the river the sacs became coated with a slimy deposit in about five days, but the integrity of the sac was not affected by it for several weeks.

These celloidin sacs and their contents were suspended in running tap water (Lake Michigan water) in a large glass receptacle containing six liters of water, one liter of which was replaced every one or two minutes. The glass tubes were cotton-stoppered, and held by burette clamps so that the sacs and the lower portion of the glass tubes were submerged.

The celloidin sacs used in the Chicago River were sealed and immersed in the water. They were protected from floating débris by being inclosed in a box, to which the water had free access.

The sacs were tested by submerging them in water, blowing into the glass tubes, and looking for air bubbles. If no air bubbles were detected, they were filled with a watery solution of egg albumin and suspended in a small jar containing water for twenty-four to forty-eight hours. The outside water was tested for albumin. After the integrity of the sacs was established by these two tests, their osmotic property was tested by solutions of salt and sulphate of magnesia.

For the collection of samples of river water it was found necessary to bring one entire sac to the laboratory daily. In the case of the Lake Michigan tap water, we were able, due to the conveniences of conducting the experiments in the laboratory, to take out small portions by means of a sterile capillary pipette.

Although the method of using permeable sacs made of celloidin has been employed for several years, so far as we are aware this technical method has not before been applied to a study of the present character. In addition to using celloidin sacs, another method was practiced in which the typhoid cultures were exposed in sacs made of vegetable parchment, such as are commonly employed in laboratories for dialyzing work. This method has been previously used by one of us,² with success, in studying the influence of certain environmental factors on growth of milk bacteria.

Dialysis occurs more or less rapidly in such sacs, and therefore any disturbance in equilibrium is quickly restored by osmosis. Dialyzable substances in the water, either inside or outside of the

¹ Bull. Johns Hopkins Hosp., 1902, 13, p. 112.

² RUSSELL, *Eighteenth Report, Wis. Agr'l. Exper. Sta.*, 1901, p. 165.

sac, would therefore be able to pass in or out. If the water outside of the sac was rich in organic matter and bacteria, and consequently contained a large amount of soluble products formed by these organisms, the osmotic pressure might result in an inflow that would continue until equilibrium was established. On the other hand, if abundant growth occurred in the sac, such as is usually observed when a small quantity of water is confined in a glass bottle, the effect of this excessive growth might be diminished by the outflow of such soluble growth products. By establishing such a state of equilibrium it would seem that the conditions within the sac must approach much more nearly to those which obtain in the flowing stream than would be possible where diffusion or osmosis is excluded.

Parchment sacs.—The parchment used in this work is purchased in the form of long tubes with a diameter of about 2.5 inches. This tubing was cut into lengths so that each sac would hold from 200 to 700 c.c. of liquid. In the experiments in the Illinois River at Averyville (H. L. R.) the sacs were made by folding about 6 inches of the tubing back and forth on itself, making at least eight thicknesses, which was then clamped tightly with strong clamps. In the Averyville experiments both ends of the sacs were closed.

In the experiments in the Drainage Canal (E. O. J.) the tubes were closed at one end by folding a number of times and then sealing tightly with a mixture of resin and tallow (8:1). The upper end of the sac was fastened to a piece of glass tubing $\frac{5}{8}$ inches in diameter and 6 inches long, the lower end of which was drawn out so that a firm union was made with the parchment by binding the moistened parchment around the tube at the point of constriction. The lower end of the parchment section of the tube (22 inches long) was sealed by turning up the end ($1\frac{1}{2}$ inches), rolling it tightly and wrapping with thread. Both the upper and lower joints were saturated with a mixture of resin and tallow. The capacity of the completed tube was 700 c.c. After about three or four days in the water, the sacs became coated with a dark, slimy, later shaggy, material, which on microscopic examination was seen to consist of a species of bryozoa. The same growth is found on all submerged planks and debris in the vicinity. This growth was most marked at Robey Street (upper end of Drainage Canal), but was seen to a less degree at Lockport (lower end).

The sacs exposed in the Illinois River at Averyville began to show small circular spots on the outside after they had been in place four or five days. These areas gradually increased in size for a time, but never became confluent. An examination showed the hyphal filaments of a pinkish fungus. In the course of about two weeks the portions of the parchment sac affected in this way softened under the development of cytolytic enzymes, so that the sacs were quite readily ruptured. After losing several sacs in this way in the

course of eleven or twelve days, the contents of the remaining sacs were transferred to new tubes after they had been immersed about a week.

When properly prepared, this parchment tubing is firm and tough, and capable of withstanding considerable rough treatment. Care must be taken, however, in selecting the sacs, as they are frequently defective along the seams. The sacs were first tested by filling and suspending in air, rejecting any which showed any signs of leakage. Their integrity was also tested in the following way: Sacs were filled with water, then immersed in cylinders filled with water, after which the whole apparatus was sterilized in the autoclave. In some cases the outside water was inoculated with typhoid bacilli, and in others the infection was made on the inside of the parchment sacs.

In carrying out the experiments in the water of the Drainage Canal, the parchment sacs were suspended within a frame covered with wire screen. This frame was made of pine strips and was four feet long, three feet wide, and three and one-half feet deep. The bottom, top, and lower five-sixths of the sides and ends were covered with wire screen; the upper six inches of the sides and ends of the box, and the sides and ends of the cover, which were six inches deep, were made of one-inch pine fencing. The top opened on hinges, and was fastened in place by hasp and padlock. The box, as a whole, was so placed that it floated with the water-level at about the lower margin of the upper solid half of the body. The part above water was thus of boards and served to protect the contents from waves made by passing vessels or by high winds.

Within the box were several cross strips about one inch above the water-level, in which, at intervals of about two inches, slots were cut to receive the glass ends of the tubes. Opposite each slot was a staple through which was passed a cord attached to the sac to hold it securely in place. To guard further against the tipping of the sacs, small strips were fastened across the slots so as effectually to prevent accidental dislodgment. At the lower end of each sac a small lead weight was attached which kept the sac in a vertical position in the water. A cord was also fastened to the lower end by which the sac was brought to the surface and its contents agitated at the time of taking samples. The sacs were open to the air at the upper end, through the glass tube, allowing free interchange of air and gases. Owing to the slight wave-motion usually present, there was a fairly constant, though slight, movement of the body of the sacs.

Two points in the Drainage Canal were selected for carrying out the experiment, one box being placed in the water at Robey Street near the mouth of the canal, and the other at Lockport near the Bear Trap Dam, twenty-eight miles from Robey Street. In all the experiments the sacs were filled with water taken from the Drainage Canal at the point where the experiment was being performed; and into the sacs thus filled typhoid bacilli were immediately introduced. Several sacs were always inoculated at the same time and in the same way, to avoid the danger of a break in the experiment through possible mischance.

Owing to the frequency of high waves in the Illinois River at Averyville, it was found necessary to submerge the sacs about six inches below the surface of the water. The sacs were placed in galvanized iron cylinders, the top

and bottom of which were covered with a coarse wire netting, to which the sacs were firmly clamped. The covers to these cylinders were padlocked, and the whole apparatus was lowered into the river at midstream, just above the Averyville wagon bridge.

Seeding the sacs with typhoid cultures.—The sacs were first filled with the respective waters taken from the different stations, and then seeded with homogeneous suspensions of fresh cultures of the different typhoid strains. The range used in the Lake Michigan and Chicago River experiments was from 500 to 2,000,000 typhoid bacilli per c.c.; in the Drainage Canal, 180 to 857,000 per c.c.; and in the waters of the Illinois River, from 540 to 20,400 per c.c. The data as to the seeding are supplied in more detail in connection with the tabular matter later presented.

It is important to determine the absolute number of typhoid organisms exposed to the influence of the river water, but it is perhaps more important to note the relation that exists between the bacteria normally present in the various waters and the typhoid bacilli introduced. The extent of typhoid seeding should therefore be studied from the relative as well as the absolute point of view.

The sewage-polluted waters of the Chicago River and the Drainage Canal contain a varying, but always a large, number of organisms, while the waters from the Lake and the Illinois River are relatively low in bacteria. From observations made on the germ-content of the river and canal, the following data as to the average bacterial content of the water at the different stations are presented:

TABLE II.

NORMAL BACTERIAL CONTENT OF WATERS TAKEN AT DIFFERENT STATIONS,
COMPARED WITH TYPHOID SEEDINGS.

STATIONS	BACTERIA PER CUBIC CENTIMETER	
	Water and Sewage Forms	Typhoid Bacilli Added.
Lake Michigan.....	68- 2,000	540-1,184,000
Chicago River.....	80,000-1,500,000	500-2,000,000
Drainage Canal (Robey Street).....	100,000- 400,000	670- 857,000
Drainage Canal (Lockport).....	100,000- 400,000	180- 360,000
Illinois River (Averyville).....	1,800- 4,000	540- 20,400

It is evident from the foregoing that the number of typhoid bacilli introduced into the respective waters was usually a large proportion of, and often surpassed, the number of bacteria of all kinds naturally found in such waters.

Method of isolating the typhoid bacilli.—Particular attention was given to the problem of isolating typhoid bacilli in the presence of sewage bacteria. Many media have been proposed for this purpose, and a number of the most promising were tested with a view to possible utilization.

One of us (F. R. Z.) made a comparative study of the value of the following methods for the detection of small numbers of typhoid bacilli in badly polluted waters, which will be the subject of a future paper:

1. Special culture methods.
 - a) Drigalski-Conradi method.¹
 - b) Drigalski-Conradi agar plates, followed by subcultures in Hiss tubes.
2. Direct agglutination method for large quantities of water.
 - a) Hagemann method.²
3. Enriching methods with agglutination.
 - a) Schepilewsky's modification of Windelbandt's method.³
 - b) Altschüler's method.⁴
4. Chemical precipitation method for large quantities of water.
 - a) Schüder.⁵

Of these the Drigalski-Conradi method, followed by subcultures in Hiss tubes, was used extensively, and gave excellent results where small quantities of water had to be used for examination. Altschüler's and Schüder's methods both proved highly efficient in the examination of large quantities of water for typhoid bacilli.

Thanks are due to Messrs. Parke, Davis & Co., of Detroit, for kindly furnishing us an antiserum of very high agglutinating power, together with cultures of their strain "O" B. typhosus, used in immunizing the horse from which this serum was obtained.

In the work on the Drainage Canal (E. O. J.) four media were chiefly used, viz.:

a) The medium of Drigalski and Conradi.¹ This was modified from the original description of these writers by leaving out the nutrose, sodium chloride, and the solution of water-free sodium carbonate, and by filtering the mixture before adding the litmus lactose solution. In this way a much clearer medium was secured which contained scarcely any flocculent precipitate after being sterilized thrice in the steam bath.

b) A modification of MacConkey's medium.⁶ At first the original MacConkey medium, as modified by Grünbaum and Hume, was used, but this was found to have very little restraining power upon the sewage bacteria, so

¹ *Ztschr. f. Hyg.*, 1902, 39, p. 283.

² *Centralbl. f. Bakt.*, 1903, 33, Apr. 22, p. 743.

³ *Ibid.*, Feb. 6, 1903, 33, p. 394.

⁴ *Ibid.*, Apr. 22, 1903, 33, p. 741.

⁵ *Ztschr. f. Hyg.*, 1903, 42, p. 317.

⁶ *Brit. Med. Jour.*, 1902, Pt. I, p. 1473.

that crystal violet solution (1 g. to 100 c.c.) was added in the proportion of 83 c.c. per liter of the medium, and the technique was further altered in the following manner: After the agar and peptone were dissolved, the medium was made 1.8 acid to phenolphthalein by adding a sodium hydrate solution, and the mass was autoclaved for five minutes at 120° C., cooled, cleared with white of egg, and filtered through cotton. This treatment prevented the formation of the flocculent precipitate which caused trouble in the original method. Finally the other ingredients were added and also the crystal violet, and the medium was tubed and sterilized once in the Arnold sterilizer for thirty minutes.

c) Horrock's glucose litmus agar.¹ Two per cent. glucose peptone agar was made in the usual manner, and sufficient aqueous solution of litmus was added to give the medium a light blue color. For use the medium is melted, cooled to 42° C. and then decinormal sodium hydrate added so that each 10 c.c. of the medium has an alkalinity of 1.8 c.c. decinormal alkali. This medium differentiates well, but the abundant gas formation impairs its usefulness for the conditions under which we worked.

d) The plating medium of Hiss. This was prepared according to the description given by the author.² Although not restraining the development of sewage forms in the way that the other media did, it proved one of the most useful methods, particularly when the plates were not too thickly crowded with sewage bacteria. Differentiation of the surface colonies was usually very sharp.

Other media, notably Elsner's potassium iodide medium³ were tested, but proved very inferior to the four media above described; these four alone were used in the routine work.

In the work on the Illinois River at Averyville (H. L. R.) the Drigalski and Conradi medium was exclusively employed, differentiation being here less of a problem than in the waters charged with sewage bacteria.

EXPERIMENTS CONDUCTED IN LAKE MICHIGAN WATER AND IN THE CHICAGO RIVER (F. R. Z.).

A series of experiments upon the longevity of the typhoid bacillus in Lake Michigan water, made during the fall of 1897 and the spring of 1898, had convinced the writer that typhoid bacilli die much more rapidly when free in nature than had been generally believed. Although the conclusions drawn were open to the criticism that in the liter flasks, used in experiments to hold the artificially infected lake water, the conditions were different from those existing in nature, the results compare very well with those obtained in our experiments with parchment and celloidin

¹ W. H. HORROCKS, *An Introduction to the Bacteriological Examination of Water* (London), 1901, p. 205.

² *Jour. Med. Resch.*, 1902, 8, p. 148.

³ *Ztschr. f. Hyg.*, 1895, 21, p. 25.

sacs suspended in Lake Michigan tap water, under the most favorable conditions for the diffusion of saprophytic products.

The experiments consisted in seeding liter flasks containing Lake Michigan tap water with varying numbers of typhoid bacilli, and studying the effects of temperature, light, and the addition of saprophytic products (sewage) upon the longevity of typhoid bacilli.

Similar experiments were undertaken with the water of the Mississippi, Missouri, and the Illinois Rivers at Grafton, between the months of October, 1901, and March, 1902, and it was found that the more polluted a water was and the more saprophytes were present, the sooner would the typhoid bacilli disappear.

The natural objection to these flask experiments, that the accumulating products of this saprophytic overgrowth were the cause of the rapid disappearance of typhoid bacilli, and that the results could not, therefore, be applied to conditions naturally existing in lake or river water, has not been justified by the results of the experiments to be related, because typhoid bacilli have died in our parchment and celloidin sacs, under the most favorable conditions of diffusion, in the natural flowing water, in as short, or even a shorter, time than they did in the same waters when contained in glass flasks at temperatures ranging from 12° to 20° C.

The present experiments give first the results with glass bottles as containers, in which diffusion of bacterial excretion products is excluded; and next, the results when diffusion of excretion products is favored by the use of parchment and celloidin sacs.

I. EXPERIMENTS WITH LAKE MICHIGAN TAP WATER.

In the first five of the following fifteen experiments with Lake Michigan tap water, glass jars or bottles were used as containers; in the next seven, parchment sacs; and in the last two, celloidin sacs, the parchment and celloidin sacs being suspended in running tap water.

Experiment 1.—Eight hundred c.c. of raw (unheated) tap water, taken from a tap in Dr. Zeit's laboratory at the Postgraduate Hospital, were placed in a glass jar, and one loopful of a twenty-four-hour agar culture of *B. typhosus* (Race 0 from Parke, Davis & Co.) was added, the seeding being

estimated by plating to be approximately 1,000,000 per cubic centimeter. The bacterial count of the water at the time of seeding was 85 per cubic centimeter. The temperature of the water varied from 7° to 16° C. Above, the jar was open to the air, which entered freely through the cotton stopper.

Samples were taken daily, after shaking the jar to insure thorough mixing, a portion of the contents being taken with a sterile pipette. Plate cultures were made in plain neutral agar, with a seeding of from 1.1.000 to 5 c.c., according to the bacterial content. These cultures were incubated at 39° C. After twenty-four to forty-eight hours the plates were carefully examined and the typhoid-like colonies cultured in Hiss's tube medium. Cultures in this medium which resembled those of the typhoid bacillus were further examined, and their identity established by the specific agglutination test of Widal, and also by further culture methods. From the tenth to the fourteenth day all the colonies appearing on the plates were examined, with negative results, and the complete disappearance of the typhoid bacillus was demonstrated. For comparison, plate cultures of the daily samples were made and incubated at 25° C., and the bacterial content per cubic centimeter (after three days), and the character of the growth, compared with that of the plate cultures at 39° C.

The results of the experiment are shown in Table III.

TABLE III.

Day of Experiment	Temperature	Bacteria per c.c. of Tap Water	Bacterial Count of Plates Incubated at 39° per c.c.	Bacterial Count of Plates Incubated at 25° per c.c.	B. typhosus + = present 0 = absent
Seeding	9° C.	85		1,737,000	+
1	14°	90	735,000	1,027,000	+
2	13°	109	711,000	1,175,000	+
3	16°	100	339,000	360,000	+
4	16°	180	71,000	154,000	+
5	9°	38	5,800	28,600	+
6	10°	80	9,300	12,400	+
7	9°	48	1,600	3,200	0
8	12°	48	5,000	4,700	0
9	12°	76	360	1,000	0
10	9°	80	78	8,000	0
11	8°	148	197	760	0
12	7°	128	41	1,260	0
13	16°	120	72	1,820	0
14	8°	110	0	2,180	0

NOTES.—On the eighteenth and twentieth days no colonies developed in plates seeded with 1 c.c. of the water, and in plates seeded with 5 c.c. only a few colonies developed at 39° C. On the seventh, eighth, and ninth days no typhoid-like colonies developed on the plates. From the tenth to the fourteenth days, inclusive, all the colonies on the plate cultures were examined.

Experiment 2.—In this experiment four loopfuls of the water of condensation were used, and the flask was placed in an earthen jar and surrounded by flowing water kept at a uniform temperature (1° 9 C.) for twelve days. The germ content was 180 per c.c. and plating after seeding with *B. typhosus*, 2,200,000 per c.c.

Plates poured with Drigalski Conradi¹ agar (about 3 mm. thick) remained open for an hour until the agar became hard. After shaking the flask containing the water to be examined, a glass rod, bent at an angle, sterilized, was dipped into the water and smeared over the first plate. Dilutions were made by smearing a second, third, and fourth plate. These plates remained open until perfectly dry (one-half to one hour), and were then incubated at 37° for twenty-four hours. All discrete blue colonies were subcultured in Hiss tubes and grown at 20°. All non-gas-forming, uniformly clouded Hiss tubes were tested by Parke, Davis & Co.'s immune serum for agglutination.

Typhoid bacilli were found by this method on the third, fifth, sixth, and seventh days, but none on the eighth or ninth days.

On the twelfth day Altschüler's² method was used. Ten grams of peptone and 5 g. of sodium chloride were added to the contents of the flask which was then incubated for twenty-four hours at 37° C. Fifty c.c. of the upper stratum of the flask contents were placed in a sterile burette, and 1 c.c. of Parke, Davis & Co.'s serum was added. This was set aside for seven hours. No precipitate was visible. One c.c. was drawn off into another burette containing 50 c.c. of sterile peptone (0.5 g.) and sodium chloride (0.25 g.) solution and a few millet-seed-sized pebbles. This burette was shaken to break up clumps and incubated at 37° for twenty-four hours. Drigalski Conradi plates were smeared and incubated as above; blue colonies were subcultured in Hiss tubes. No non-gas-forming, uniformly clouded Hiss tubes.

Experiment 3.—Fresh feces of a healthy person were stirred into 1,000 c.c. of raw tap water until the turbidity was such that print became indistinct when viewed through the solution in a 1,000 c.c. cylindrical graduate. This mixture was subjected to the same conditions of seeding and temperature as in the last experiment. The immediate plating in gelatin was 5,800,000 per cubic centimeter. Examination, as in the last experiment, showed typhoid bacilli on immediate plating, but none on the first, third, or eighth days thereafter.

As a control for this experiment two 1,000 c.c. flasks were prepared with human excreta as above. One (A) was seeded with four loopfuls of the usual culture of *B. typhosus*; the other (B) received no typhoid bacilli until it had been kept twenty-four hours at a temperature of 19° C., when it was seeded with 1:1,000 loop of the same culture as Flask A, after which both flasks were examined by Schüder's method.³

To each flask were added 10 c.c. of a 7.75 per cent. solution of hyposulphite of soda and 10 c.c. of a 10 per cent. solution of nitrate of lead. After standing for twenty-four hours the clear fluid was decanted, 7 c.c. of a 100 per cent. solution of hyposulphite of soda added to the sediment, and the flask shaken. After standing a short time, the insoluble particles settled. Drigalski-Conradi smears were made and examined as in the preceding experiments, from the upper clear strata.

In Flask A, seeded with four loopfuls of typhoid culture twenty-four hours before precipitation, typhoid bacilli were absent, while in Flask B, seeded with only 1:1,000 loop just before precipitation, the *B. typhosus* was found.

¹ *Ztschr. f. Hyg.*, 1902, 39, p. 283.

² *Centrbl. f. Bakt.*, 1903, 33, p. 741.

³ *Ztschr. f. Hyg.*, 1903, 42, p. 317

Schüder's method is of much value, as it enables us to precipitate all the bacteria in a larger quantity of water, and it allows the detection of typhoid bacilli in water highly polluted with saprophytes. Although the growth of saprophytes is much inhibited, typhoid colonies were numerous in plates from Flask B, despite the fact that the seeding of typhoid bacilli was only 1/1,000 loop with nearly 6,000,000 per c.c.

Experiment 4.—In this experiment sterilization for thirty minutes at 120° in the autoclave preceded the seeding, and the flask was kept in flowing tap-water in a uniform temperature (19° C.) for forty days. The results are shown in the following table:

TABLE IV.

Direct plating - - - - -	1,500,000	typhoid bacilli per 1 c.c.
After 5 days - - - - -	350,000	" " " "
After 10 days - - - - -	110,000	" " " "
After 15 days - - - - -	10,000	" " " "
After 20 days - - - - -	30	" " " "
After 25 days - - - - -	12	" " " "
After 30 days - - - - -	0	" " " "
After 35 days - - - - -	0	" " " "
After 40 days - - - - -	0	" " " "

Experiment 5.—In this experiment a four ounce bottle stopped with a sterile cotton plug was used instead of the liter flask, and the tap water was rendered germ-free by filtration through sterilized porcelain (unglazed). The germ content previous to filtration was 100 per cubic centimeter. Cultures of 5 c.c. of the filtered tap water in bullion remained sterile. To 100 c.c. of this sterilized tap water was added 1 c.c. of a 1:10,000 dilution in sterile salt solution of a twenty-four hour agar culture of *B. typhosus* Race No. 500.2 (a culture obtained from the Johns Hopkins Pathological Laboratory). This seeding was estimated by plate methods to be about 500 typhoid bacilli to 1 c.c. The bottle containing the infected water was placed in a thermostat maintained at a temperature of about 20° C. Free entry of air was allowed through the cotton plug; light was excluded.

The bottle was removed daily, and, after shaking gently to insure even seeding, plate cultures in agar-agar were seeded with 1 c.c. of the infected water. These cultures were incubated from twenty-four to forty-eight hours, and the typhoid-like colonies were then subcultured in Hiss tubes and identi-

TABLE V.

Day of Experiment	Amount of Water Plated	No. of Typhoid Bacilli Found	Estimated No. in Bottle	<i>B. typhosus</i> + = present 0 = absent
Seeding	1 c.c.	500	50,000	+
1	1	4	396	+
2	1	1	98	+
3	15	2	11	+
4	30	1	3	+
5	15	0	0	0
6	10	0	0	0
7	5	0	0	0
8	5	0	0	0
9	5	0	0	0
10	12	0	0	0

fied by means of further culture, and the Widal reaction. As the typhoid organisms disappeared, larger amounts of water were used for seeding. During the last three days of the experiment all the water remaining in the bottle was plated out, but no typhoid organisms were found. The results are presented in Table V.

Instead of the glass containers, parchment sacs were used in the next seven experiments.

Experiments 6 and 7.—In these experiments, where the bacterial count before the seeding was estimated to be 2,000 per cubic centimeter, and from a few hundred to a few thousand, respectively, the 800 c.c. of tap water was seeded with a small loopful of a twenty-four-hour culture of *B. typhosus*, Race "y," received from Dr. E. O. Jordan, giving a seeding of about 540 000 in the one case and of 450,000 in the other. The parchment sacs were subjected, in both experiments, to the same conditions as those surrounding the glass container in Experiment 1, already described. Samples of the infected water were taken daily and subjected to the usual cultural and agglutination tests. Typhoid organisms were found daily up to the seventh day in Experiment 6, and up to the fifth day in Experiment 7, but none thereafter. In both cases there was a marked change in the character of the plates in the latter part of the first week. In Experiment 7 a single culture on the fifteenth day showed the presence of only a few saprophytic organisms.

Experiment 8.—This was in all respects as Experiment 1, except that a parchment sac was used in place of the glass container. At the end of this experiment the parchment sac was examined and found to be intact. It appeared to be as strong as when first put into the water, and there was little or no deposit on its surface. The results of this experiment are shown in Table VI.

TABLE VI.

Duration of Experiment in Days	Bacterial Count per c.c. at 25° C.	Bacterial Count per c.c. at 39° C.	Ratio Growth at 25°-39° C.	<i>B. typhosus</i> + = present 0 = absent
Seeding	1,112,000			+
1	1,035,000			+
2	964,000	372,000	2.59	+
3	855,000	576,000	1.46	+
4	616,000	553,000	1.11	+
5	536,000	316,000	1.69	+
6	237,000	205,000	1.16	+
7	103,000	201,000	0.51	+
8	134,000	197,000	0.68	0
9	72,000	23,000	3.10	0
10	32,000	4,400	7.27	0
11	13,000	3,400	3.82	0
12	7,100	74*	9.59	0
13	6,000	34*	17.64	0
14	10,600	100*	10.60	0

NOTES.—On the twelfth, thirteenth, and fourteenth days all the colonies on the plates were examined; *Bacillus typhosus* was not found.

From the first to the fifth day, inclusive, the plates resembled pure cultures of *Bacillus typhosus*, which was recovered with ease.

On the sixth, seventh and eighth days the plates showed mixed cultures of *Bacillus typhosus*, which was secured with increasing difficulty.

From the ninth to the fourteenth day, inclusive, the cultures were saprophytic in character, and the typhoid bacillus could not be found, despite repeated and exhaustive efforts to secure it.

Experiment 9.—This experiment was undertaken as a control to the preceding, the only difference being that the parchment sac in the earlier experiment was surrounded by flowing water, while in Experiment 9 it was suspended in an empty glass cylinder so that the surface of the parchment was freely exposed to the air on all sides. In order to secure temperature conditions similar to those of Experiment 8 the glass cylinder was placed in a large receptacle and surrounded with running tap water. There was free access of air also through the cotton stopper, and the experiment was conducted in the diffuse light of the laboratory. The results are shown in Table VII.

TABLE VII.

Duration of Experiment in Days	Bacterial Count of Plates Incubated at 39° C.	Bacterial Count of Plates Incubated at 25° C.	Bacillus typhosus + = present 0 = absent
Seeding		635,000	+
1		748,000	+
2	711,000	628,000	+
3	340,000	1,836,000	+
4	419,000	2,117,000	+
5	158,000	1,117,000	+
6	142,000	253,000	0
7	13,800	83,000	0
8	316 *	45,000	0
9	1,100 *	11,600	0
10	550 *	2,600	0
11	840 *	6,400	0
12	No growth	11,000	0
13	15 **	5,500	0
14	13 **	8,600	0

NOTES.—On the third, fourth, and fifth days the plate cultures showed a large number of fluorescent organisms. On the sixth to the ninth days inclusive, the fluorescent organisms were numerous, but decreasing in number.

* On the ninth, tenth, and eleventh days all the typhoid-like colonies on the plates were examined.

** On the thirteenth, and fourteenth days all the colonies on the plates were subcultured and examined, but the typhoid bacillus could not be found.

RESULTS.—An overgrowth of saprophytes occurred early in the course of the experiment, and the vital concurrence of these organisms, as well as the action of their products, must be regarded as factors in the death of the typhoid bacillus.

Experiment 10.—In this experiment 800 c.c. of raw tap water, which gave 180 colonies per centimeter in gelatin plates, were placed in a cotton-stoppered parchment sac and seeded with four loopfuls of the water of condensation of a twenty-four-hour culture of *B. typhosus*, Race "O," Parke, Davis & Co. The sac was suspended in running tap water in a large earthenware jar, the temperature of the rapidly flowing water being 1°5–2°5C. The contents of the sac were examined by Drigalski-Conradi plates and Hiss tubes, with the result of finding typhoid bacilli on the second, fourth, fifth, and sixth days, and with negative results on the seventh and eighth. The Schüder precipitation method was employed as described in Experiment 3, with the balance of the sac contents, on the ninth day, with negative results.

Experiment 11.—A parchment sac was prepared, seeded, and examined as in the preceding experiment. Plating after seeding in gelatin showed 2,300,000 colonies per 1 c.c. Typhoid bacilli were found on the second, fifth, sixth,

and seventh days, but none on the eighth or ninth. On the tenth day Alt-schüler's enriching method was employed, as in Experiment No. 2, with negative results.

Experiment 12.—Eight hundred c.c. of tap water were sterilized by heating for twenty minutes in the autoclave at a temperature of 110° C. The sac was then placed beside the one used in Experiment 8 and subjected to the same conditions. All the other conditions of the experiment, and the method of examining the sac contents for typhoid bacilli, were as in Experiment 8. The result was a steady decrease in the number of typhoid organisms found, but they were still present on the fifteenth day, when the experiment was discontinued. The results are tabulated in Table VIII.

TABLE VIII.

Duration of Experiment in Days	Bacterial Count of Plates Incubated at 39° C. per c.c.	Bacterial Count of Plates Incubated at 25° C. per c.c.	Bacillus typhosus + = present 0 = absent
Seeding		1,184,000	+
1	450,000	618,000	+
2	416,000	716,000	+
3	363,000	510,000	+
4	350,000	904,000	+
5	332,000	814,000	+
6	253,000	366,000	+
7	292,000	316,000	+
8	356,000	474,000	+
9	94,800	94,800	+
10	12,000	83,200	+
11	16,000	150,000	+
21	3,120	28,700	+
13	840	64,000	+
14			+
15	1,000	25,000	+

Experiment 13.—In this experiment a four-ounce glass-stoppered bottle was used. A sterilized salt solution was made by adding 0.6 per cent. of common salt to the tap water and heating at 20° C. in the autoclave. To 100 c.c. this, 1/10,000 of a large loopful of a twenty-four-hour culture of *B. typhosus* (Race No. 500.2, obtained from the Johns Hopkins Pathological Laboratory) was added, the seeding being estimated by plate cultures to be 900 per cubic centimeter. The bottle was placed in the ice-chamber of the refrigerator, the

TABLE IX.

Day of Experiment	Amount of Water Plated	No. of Typhoid Bacilli per c.c.	No. in Bottle Estimated	Bacillus typhosus + = present 0 = absent
Seeding	1 c.c.	900	90,000	+
1	1 c.c.	804	79,500	+
2	1 c.c.	426	41,700	+
3	1 c.c.	326	31,600	+
4	1 c.c.	266	25,500	+
5	1 c.c.	158	15,000	+
6	1 c.c.	145	14,000	+
7	1 c.c.	140	13,000	+
8	1 c.c.	100	9,200	+
9	1 c.c.	80	7,500	+
10	1 c.c.	75	6,800	+

temperature varying from 1° to 8° C. A glass stopper was used, but about 10 c.c. of air were inclosed and renewed daily as the bottle was opened. Light was excluded. Plate cultures were made daily, and as the typhoid bacilli became fewer in number, all the colonies on the plate were examined. The typhoid bacillus was still found on the tenth day, when the experiment was discontinued. The results are presented in detail in Table IX.

Experiment 14.—In this experiment celloidin sacs having a capacity of 30 c.c. each were used in place of parchment sacs. In this case the bacterial count of the tap water at the time of seeding was 68 per cubic centimeter, and the seeding was estimated to be 1,000,000 per cubic centimeter. The infected water was distributed by means of a sterile pipette to the celloidin sacs in amounts of about 20 c.c. The experiment was in all other respects similar to No. 8. The results are presented in Table X.

TABLE X.

Duration of Experiment in Days	Bacterial Count of Plates Incubated at 25° C. per c.c.	Bacterial Count of Plates Incubated at 39° C. per c.c.	Bacillus typhosus + = present 0 = absent
Seeding	1,155,000		+
1	883,000	814,000	+
2	790,000	946,000	+
3	1,146,000	717,000	+
4	737,000	565,000	+
5	320,000	53,600	+
6	292,000	7,000	+
7	514,000	4,000	+
8	558,000	35,600	0
9	600,000	3,800***	0
10	569,000	2,600*	0
11	711,000	3,960	0
12	87,000	182**	0
13	4,030	28**	0
14	3,900	23**	0

NOTES.—On the fourth day numerous fluorescent forms appeared in the plates, especially those incubated at low temperature.

* All the colonies appearing on one-fourth the plate examined with negative results.

** All the colonies appearing on the plates examined with negative results.

*** Negative results obtained by special method. All of the water was removed from one of the capsules, antityphoid serum added in the proportion of 1:100, and the whole placed in a sterile burette for sedimentation. At the end of twenty-four hours the lower portion, about 1 c.c., was removed and plated in nutral agar. The colonies which developed were examined, but the typhoid bacillus was not found.

Experiment 15 differed from the preceding only in that the tap water was sterilized before seeding (as in Experiment 12), and the results confirmed those obtained in Experiment 12, in which a parchment sac was used to contain the sterilized water.

The following eleven experiments, similar in general to those already described with Lake Michigan water, were conducted with the water of the Chicago River:

Experiment 16.—This experiment corresponded in all respects to Experiment 5 above, except that the water of the Chicago River was used instead

of that of Lake Michigan, the germ content before filtration being estimated to be 1,500,000 per cubic centimeter. The results are presented in detail in Table XI.

TABLE XI.

Duration of Experiment in Days	No. of Typhoid Colonies in 1 c.c.	Estimated No. of Typhoid Bacilli in Bottle	Bacillus typhosus + = present 0 = absent
Seeding	496	49,600	+
1	1	99	+
2	0	0	0
3	0	0*	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0

*On the third day a number of saprophytic organisms appeared in the plate cultures. A control experiment consisting of a culture of 5 c.c. of the filtered water used in beginning the experiment showed that the filtration had not secured an absolutely germ-free water. Diligent search failed to show the presence of the typhoid organism after the first day.

This experiment indicates that if but a small number of the typhoid organisms are present in the river water, their death is a matter of hours rather than of days, and may be independent of the presence of saprophytes in large numbers.

To determine whether the amount of water used in seeding the plates had any deterring effect upon the growth of organisms in the plate cultures, cultures were made from two preparations: (1) 100 c.c. of raw filtered water of the Chicago River, to which was added 1/10,000 part of a loopful of a twenty-four-hour agar culture of *B. typhosus*; (2) 100 c.c. of sterile salt solution seeded with typhoid as in (1). Plate cultures were made of 1 c.c. of each mixture, and after forty-eight hours' incubation at 39° C. the number of colonies was found to be as follows: (1) 496 per cubic centimeter; (2) 500 per cubic centimeter. Repetition of the experiment gave a similar result, so that it was evident that small amounts of the water, when added to nutrient media, had no marked deterrent effect upon the growth of the typhoid bacillus in plate cultures.

Experiment 17.—In this experiment the bottle containing the filtered Chicago River water was placed in the ice-chamber of the refrigerator, the temperature being 1° to 8° C. Glass stoppers were used, but about 10 c.c. of

air was inclosed, and renewed in part daily as the bottles were opened. The bacterial content of the water was estimated to be 2,000,000 per cubic centimeter. All the other conditions were as in the preceding experiment.

The death of the typhoid bacillus was rapid in the river water at this temperature, but not so rapid as in the preceding experiment at a temperature of 20° C. The specific bacillus was not found, however, after the fourth day. Its complete disappearance was shown by the comprehensive nature of the examination. The results are given in Table XII.

Experiment 18.—The water, after filtration and seeding as in the preceding experiment, was distributed by means of a sterile pipette in amounts of 5 c.c. each to sterile test-tubes. These were subjected to the temperature of the air outside the laboratory (below 0° C.). In the course of a few minutes the water in the tubes was frozen, and remained so except when melted for examination. Sterile stoppers of cotton were used, admitting free access of air. Light was excluded.

One tube was taken daily and the ice melted by immersing the tube for a few minutes in a water-bath heated to 35° C. One c.c. of the water was then plated in neutral agar, and the typhoid organism sought by the methods already described. It was found that even at temperatures below 0° C. the typhoid bacillus dies rapidly in the filtered water of the river, but two organisms being found on the first day and not one later. Its complete disappearance is shown by the failure to find the specific organism in plate cultures of all the water remaining in the tubes, which contained at the beginning several thousand typhoid organisms.

TABLE XII.

Day of Experiment	Amount of Water Plated	No. of Typhoid Bacilli on Plates	Estimated No. in Bottle	B. typhosus + = present 0 = absent
Seeding	1 c.c.	1,080	108,000	+
1	1	408	40,392	+
2	3	260	8,516	+
3	5	120	2,280	+
4	5	24	432	+
5	5	0	0	0
6	5	0	0	0
7	5	0	0	0
8	5	0	0	0
9	5	0	0	0
10	60	0	0	0

Experiment 19.—To 800 c.c. of the water of the Chicago River, taken at the Ashland Avenue bridge (estimated bacterial content 2,000,000 per cubic centimeter), was added a large loopful of twenty-four-hour agar culture of *B. typhosus* strain "x," received from Professor Jordan. A parchment sac, containing the infected water, was placed in the river at the Ashland Avenue bridge, being placed for protection in a box to which the river water had free

access, but which excluded the light. The temperature of the surrounding water was found to vary from 12° to 14° C. There was free access of air through the cotton stopper in the glass tube of the capsule.

Samples of the water were taken daily, plate cultures made in neutral agar, and, after incubation for twenty-four to forty-eight hours at 39° C., the typhoid colonies and those resembling them were subcultured in Hiss tubes, and the identity of the organisms was established by the usual cultural and agglutination tests. The typhoid bacillus was recovered with comparative ease on the first and second days, but with difficulty on the third. On the fourth day the sac was found to have a small hole in it, and the experiment was discontinued.

Experiments 20 and 21 were repetitions of Experiment 19. The typhoid bacillus was recovered up to the second and third days respectively, but not later, the experiment being continued for ten days in each case.

Experiment 22.—Race "O" from Parke, Davis & Co. was used in seeding the river water in this experiment. The estimated bacterial content before seeding was estimated to be 80,000, and the seeding to be 20,000 per cubic centimeter. The temperature of the water surrounding the parchment sac was taken daily and found to agree with that of the water in the sac, varying from 8° to 4° C. The other conditions of the experiment were as in the preceding three.

Plate cultures were made in neutral agar, using a seeding of about 1/100 c.c. The typhoid-like colonies were subcultured in Hiss tubes, and their identity was established by culture methods and the Widal test. To obviate the objections which might be made on account of the very small amounts of water used in seeding, a large number of plates were prepared, and use was made of the special method of Schepilewsky.

The typhoid organism was found with ease on the first day, and with difficulty on the second and third, after which it could not be found. Use of a special method on the fourth, seventh, and tenth days gave negative results.

Experiment 23.—This was designed as a control experiment to determine the fate of the typhoid organisms when placed under the influence of the polluted river water, but not in direct contact with the bacteria thereof. To 800 c.c. of tap water (Chicago supply), sterilized by heating for thirty minutes in the autoclave at a temperature of 120° C., was added a loopful of a twenty-four-hour culture of the typhoid organisms, as in the preceding experiments, the seeding being estimated to be about 1,500,000 per cubic centimeter. The infected water was then introduced into the parchment sac, which was subjected to the same conditions as in Experiment 19.

The typhoid bacillus was found on the first and second days, but not later.

Experiment 24.—One twenty-fifth of a small loopful of a forty-eight-hour culture of the typhoid organism (Race "O," Parke, Davis & Co.) was added to 500 c.c. of the river water, which was estimated at the time of seeding to contain 640,000 water and sewer organisms per cubic centimeter. After agitating to insure uniform seeding, the infected water was distributed to celloidin capsules in amounts of 20 c.c. each. These sacs were sealed and immersed in the water of the Chicago River at the Ashland Avenue bridge. The temperature of the water ranged from 1° to 4° C. The capsules were inclosed in

a box, to which the water had free access, but which excluded light. This protected them from ice.

Search was made as in Experiment 22, already described, for the typhoid bacillus, with the result that the organism was recovered with ease immediately after seeding, and twenty-four hours later, but not thereafter. On the fifth and the ninth days all the water in a capsule was taken and antiserum added in the proportion of 1:100. This mixture was placed in a sterile burette, and after sedimentation for twenty-four hours the lowest portion was drawn off and plated. The typhoid-like colonies were tested in the usual manner, with absolutely negative results.

Experiment 25.—In this the river water was filtered, and the celloidin sac containing it was suspended in a glass jar in about 400 c.c. of the river water, which was estimated to contain 500,000 organisms per cubic centimeter. The typhoid bacilli were thus subjected, not only to the action of the toxic agent in the filtered water of the sac, but also to that produced in the unfiltered water surrounding it. The results are presented in Table XIII:

TABLE XIII.

Day of Experiment	No. of Typhoid Bacilli per c.c.	Bacillus typhosus + = present 0 = absent
Seeding	572	+
1	330	+
2	110	+
3	49	+
4	15	+
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Experiment 26.—This differed from the preceding experiment only in that normal salt solution, sterilized as in Experiment 23, was used, and the water in the glass jar in which the celloidin sac containing the infected salt solution was suspended was also rendered germ-free by filtration through a sterile unglazed porcelain filter. The typhoid bacilli were thus subjected to the action of the toxic agents in the water passing through the walls of the celloidin sac. The temperature was about 20° C. The disappearance of the

TABLE XIV.

Day of Experiment	No. of Colonies of Typhoid Bacilli	Bacillus typhosus + = present 0 = absent
Seeding	900	+
1	860	+
2	2	+
3	0	0
4	0 *(infected)	0
5	0 **	0
6-10	0	0

* A few saprophytes appeared on the plates.

** The cultures showed an overgrowth of saprophytes.

typhoid bacillus was sufficiently rapid to justify the conclusion that the toxic agent passed rapidly through the walls of the celloidin sac. The results appear in Table XIV.

In addition to the above experiments with the water of Lake Michigan and the Chicago River, three experiments were conducted to determine the longevity of *B. dysenteriae* Shiga in these waters.

Experiment 27.—To 500 c.c. of raw (unheated) water of Lake Michigan, taken from the tap in Dr. Zeit's laboratory and estimated to contain 200 organisms per cubic centimeter, was added 1 c.c. of 1:100 dilution in sterile salt solution of a twenty-four-hour agar culture of *B. dysenteriae* Shiga, Race "Ridgely."¹ This was estimated by culture methods to give a seeding of approximately 22,000 bacilli per cubic centimeter. After agitation to insure even seeding, the water was distributed to the celloidin sacs in amounts of 20 c.c. each, by means of a sterile pipette.

Cultures were made daily in agar, and the dysentery organism was isolated by the method of Flexner. Colonies which appeared in the second twenty-four hours were subcultured on slanted glucose litmus agar. The identity of the *B. dysenteriae* was established by culture methods and by means of its reaction with antidysenteric serum.

Large numbers of the dysentery organism were recovered on the first day, a few on the second, and none at all on the third day. Later search showed their entire disappearance. Special methods (agglutination with antidysenteric serum in a manner similar to the method of Schepilewsky for the isolation of the typhoid bacillus) gave negative results on the fifth, seventh, and tenth days.

Experiment 28.—Eight hundred c.c. of the water of the Chicago River were seeded with a small loopful of *B. dysenteriae* Shiga, Race K-72², giving an estimated seeding of about 700,000 per cubic centimeter. The germ content of the water before seeding was estimated to be about 80,000 per cubic centimeter. After agitation the infected water was placed in a parchment sac and suspended in the Chicago River at Ashland bridge, as in Experiment 19.

The method of search for the dysentery bacillus and the result of the experiment were the same as in the preceding one, except that in this case two colonies of the dysentery organism were recovered on the third day.

Experiment 29.—Raw water of the Chicago River, estimated to contain 640,000 water and sewerage organisms per cubic centimeter, was used in this experiment. Five hundred c.c. were seeded with 1:100 of a small loopful of a twenty-four-hour culture of the dysentery bacillus, Race "Ridgely" being used, giving a seeding of 22,000 per cubic centimeter. The infected water was distributed to celloidin capsules, which were sealed and immersed in the river as in the other experiments.

¹A subculture of a dysentery organism isolated from a case of "summer diarrhea" at the Thomas Wilson Sanitarium, Mount Wilson, Baltimore county, Md., during the summer of 1903. The type is now distinguished as the mannite-fractor form of the dysentery organism.

²A subculture of a culture of the dysentery bacillus kept at the pathological laboratory of Johns Hopkins University, said to be a subculture of the original organism isolated by Shiga from a case of epidemic dysentery in Japan. This organism is now distinguished as the non mannite-fractor type of the dysentery organism.

Search was made for the dysentery organism as in the two preceding experiments, the bacillus being recovered on the first day but not later. Special methods were used on the seventh and ninth days, without positive results.

TABULATED SUMMARY OF EXPERIMENTS.

TABLE XV.

A. LONGEVITY OF *BACILLUS TYPHOSUS* IN LAKE MICHIGAN TAP WATER.

No. of Experiment	Container	Kind of Water in Container	No. of Saprophytes per 1 c.c.	No. of Typhoid Bacilli per 1 c.c.	No. of Days Examined	Last Day on Which Typhoid Bacilli Were Found
1.....	Glass recept.	Raw	85	1,000,000	14	6
2.....	"	Raw	180	4 loops full to 1,000 c.c.	12	7
3.....	"	Raw with excreta	5,800,000	4 loops full to 1,000 c.c.	8	Immediate plating only
4.....	"	Sterilized	0	1,500,000	40	25
5.....	"	Filtered	0	500	10	4
6.....	Parchment sac suspended in flowing tap water	Raw	2,000	540,000	10	7
7.....	"	Raw	200-2,000	450,000	15	5
8.....	"	Raw	85	1,000,000	14	8
9.....	"	Sac suspended in air	85	1,000,000	14	5
10.....	"	Raw	180	3,000,000	9	6
11.....	"	Raw	180	2,300,000	10	7
12.....	"	Sterilized	0	1,000,000	15	15
13.....	Bottle, 4-oz.	Sterile salt solution 0.6%	0	1,080	10	10
14.....	Celloidin sacs suspended in flowing tap water	Raw	68	1,000,000	14	7
15.....	"	Sterilized	0	1,000,000	15	15

B. LONGEVITY OF *BACILLUS TYPHOSUS* IN CHICAGO RIVER WATER.

No. of Experiment	Container	Kind of Water in Container	No. of Saprophytes per 1 c.c.	No. of Typhoid Bacilli per 1 c.c.	No. of Days Examined	Last Day on Which Typhoid Bacilli Were Found
16.....	Bottle	Filtered 20° C.	1,500,000 before filt.	500	10	1
17.....	Bottle	Filt. 1-8° C.	2,000,000 before filt.	1,080	10	4
18.....	Test tubes	Filtered, kept frozen	1,000,000 before filt.	600	10	1
19.....	Parchm. sac susp. in river	Raw	2,000,000	2,000,000	4	3
20.....	"	Raw	2,500,000	1,500,000	10	2
21.....	"	Raw	2,500,000	2,000,000	10	3
22.....	"	Raw	80,000	20,000	10	3
23.....	"	Sterilized	0	1,500,000	10	2
24.....	Celloidin sacs susp. in river	Raw	640,000	11,000	10	1
25.....	"	Filtered	0	572	10	4
26.....	"	Sterilized salt solution 0.6%	0	900	10	2

C. LONGEVITY OF DYSENTERY BACILLI IN LAKE MICHIGAN TAP WATER AND CHICAGO RIVER.

No. of Experiment	Container	Kind of Water in Container	No. of Saprophytes per 1 c.c.	No. of Shiga Bacilli per 1 c.c.	No. of Days Examined	Last Day on Which Dysentery Bacilli Were Found
27.....	Celloidin sacs suspended in running tap water	Tap water	1,000,000	22,000	10	2
28.....	Parchm. sac susp. in river water	Raw river water	80,000	700,000	10	3
29.....	Celloidin sacs susp. in river water	Raw river water	640,000	22,000	10	1

EXPERIMENTS CONDUCTED IN THE CHICAGO DRAINAGE CANAL (E. O. J.).

The data of the more representative individual experiments are presented in tabular form, and the results of all the experiments are summarized. Some general statements regarding method of experimentation must first be made.

A suspension in sterile water of twenty-four-hour-old agar growths was used for inoculating the sacs, and in each case a numerical determination was made to ascertain the number of bacilli present in one cubic centimeter of the contents of the sacs, immediately after inoculation. The character of the sewage into which the bacteria were introduced was also determined bacterially, and in some cases chemically also. The composition of the fluid in the Chicago Drainage Canal is that of a dilute sewage, as shown by the following analyses:

	APRIL-JUNE, 1900	
	At Mouth of Canal (Kedzie Ave.)—Average of 9 Weekly Determinations	Bear Trap Dam at Lockport—Average of 11 Weekly Determinations
Total residue on evaporation.....		196
Dissolved residue on evaporation.....		183
Chlorin.....		12.7
Oxygen consumed (total).....		6.27
Oxygen consumed (dissolved).....		4.97
Nitrogen as free ammonia.....	1.31	1.33
Nitrogen as albuminoid ammonia.....	499.499	0.347
Bacteria per cubic centimeter.....	1,332,000	1,167,000

The amount of water flowing over the Bear Trap Dam at Lockport ranged from 250,000 to 290,000 cubic feet per minute, and averaged about 275,000 (October 1 to November 9, 1903).

The following analyses were made during the conduct of the experiments:

	Drainage Canal at Robey St.	Drainage Canal, Bear Trap Dam, Lockport	
	Oct. 9, 1903	Oct. 12, 1903	Oct. 14, 1903
Total nitrogen (Kjeldahl).....	1.539	2.254	1.448
Nitrogen as free ammonia.....	0.839	1.174	1.302
Chlorin.....	13.3	12.0	12.0

The changes that occur in the water in the sacs (uninfected) are to a considerable extent independent of the composition of the canal water in which the sacs are suspended. The nature of these changes is shown in the following instances:

	A Comp. of Canal Water (Lockport)	B Comp. of Water in Cont. Sacs Filled 3 Days Prev. with Canal Water, (A)	C Initial Comp. of Canal Water	D (C), after Re- maining in Sacs 12 Days
Total N. (Kjeldahl)....	2.254	1.72	1.539	1.809
Albuminoid ammonia...	0.476	0.358	0.502	0.548
Free ammonia.....	1.174	1.484	0.8388	0.524
Oxygen consumed....	104.0	57.0	88.0	52.6
Chlorin.....	12.0	12.0	13.3	9.91

In the early experiments the numbers of typhoid bacteria introduced ranged from 500 to about 15,000 per cubic centimeter, but in the later experiments much larger numbers of bacteria were introduced. In one case as many as 857,000 per cubic centimeter were inoculated into the contents of a sac. Before beginning the experiments it was anticipated that typhoid bacilli would be found in considerable numbers on the third and fourth days, and consequently no examination of the contents of the sacs was made until after several days had elapsed. But the negative outcome of these early experiments showed that the death of the typhoid bacilli took place sooner than had been conjectured; and in the later tests it was found that the greatest mortality occurred during

the first forty-eight hours. The samples of water from the infected sacs were collected with sterilized 50 c.c. pipettes, transferred to sterilized collecting bottles, and brought immediately to the laboratory, where they were plated in the various media. It was found that the best results in differentiation were obtained when the plates were incubated twenty-four to forty-eight hours.

The colonies were usually examined after twenty-four hours, a selection made, and then the plate was returned to the incubator, and a second examination made twenty-four hours later. Except in the case of those samples removed from the sacs within the first few hours after inoculation, when the number of typhoid colonies was very large, an attempt was always made to pick off all typhoid-like colonies developing on each plate. Within three or four days after the inoculation of the sac, however, the number of typhoid-like colonies always diminished so greatly that colonies bearing a more or less remote resemblance to typhoid colonies had to be taken for examination. The colonies thus selected as showing the closest resemblance to typhoid colonies were transferred to tubes of glucose agar, and these tubes were incubated for forty-eight hours. Those in which gas production occurred were at once discarded, and from the tubes retained inoculations were made into milk.

A forty-eight-hour growth in milk at 37° C. eliminated a considerable proportion of those cultures that passed the glucose agar test. Those that gave a typhoid-like growth in milk were inoculated into dextrose litmus broth and into gelatin—a procedure which effected some further reduction in the number of typhoid-like colonies, although not nearly so many were thrown out as in the glucose agar and the milk tests.

Parallel cultures were made in each medium with the strains of typhoid bacilli used in the experiment, and only those cultures showing wide and unmistakable divergence from the parent stock were rejected.

Finally, the bacteria that passed all the cultural tests were examined in regard to their agglutinability, a serum (from the rabbit) being used which agglutinated the homologous strain in

a 1:1,000 dilution. No instance was found where a bacillus, that had passed all the cultural tests enumerated above, failed to agglutinate in an appropriate dilution.

Many colonies that were picked off because they presented a typhoid-like appearance on the plates afterward proved not to be *Bacillus typhosus*, but were found to belong to the fluorescent group of bacteria. By far the larger number of the organisms that passed the glucose agar test and were afterward rejected belonged to this class.

It was considered important to determine to what extent the lodgment of bacteria on the walls of the sacs was responsible for the disappearance of bacteria from the water in these sacs. Accordingly several sacs had the walls carefully swabbed with a pledglet of sterile cotton on the end of a rod, and samples of the contents of these sacs were taken before and after the swabbing. The counts showed in some cases a slight increase in numbers, indicating that some bacteria had become attached to the walls of the sacs and were dislodged by the swabbing process, but in no case was such increase very marked.

One of these comparisons of the colony count before and after swabbing is given in the following table :

TABLE XVI.
DRAINAGE CANAL AT LOCKPORT—COMPARISON OF SAC L₁ BEFORE AND AFTER SWABBING.

SAC No.	TIME AFTER INOCULATION	NO. OF COLONIES PER C.C.		COLONIES FISHED	TYPHOID COLONIES FOUND
		Medium	48 hr. count		
L ₁	3 days	Drigalski	3	8)	0)
		Horrocks	36	4) 20	0) 0
		Hiss	232	8)	0)
L ₁ After swabbing	3 days	Drigalski	11	10)	0)
		Horrocks	28	10) 20	0) 0
		Hiss	285	10)	0)

A tabulated summary of typical experiments conducted in the manner above described is contained in the following tables: (XVII–XXIII).

TABLE XVII.

DRAINAGE CANAL AT LOCKPORT.

Initial colony count sewage bacteria	- - - -	110,000
Initial colon bacilli	- - - - -	4,000-6,000
Initial streptococci	- - - - -	1,000

B. typhosus, Strain (x), was added to this sewage in such numbers that each sac contained approximately 12,000 typhoid bacilli per cubic centimeter.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found	
1 day.....	B ₁	...	Drigalski	...	40	60	0
1 day.....		...	Horrocks	...	20		
2 days.....	B ₂	...	Drigalski	...	40	70	0
2 days.....		...	Horrocks	...	30		
3 days.....	"	1.0	Drigalski	...	4	19	0
3 days.....	"	0.1	Drigalski	...	3		
3 days.....	"	1.0	Horrocks	...	2	3	0
3 days.....	"	0.1	Horrocks	...	3		
3 days.....	"	1.0	MacConkey	...	7	24	0
5 days.....	"	1.0	Drigalski	86	8		
5 days.....	"	1.0	Horrocks	50	8	45	0
5 days.....	"	1.0	MacConkey	166	8		
7 days.....	"	1.0	Drigalski	120	25	49	0
7 days.....	"	1.0	Horrocks	170	20		
9 days.....	"	1.0	Neutral agar	...	20	7	0
9 days.....	"	1.0	Drigalski	...	12		
9 days.....	"	1.0	Horrocks	...	7	10	0
9 days.....	"	1.0	MacConkey	...	10		

During the conduct of this experiment, October 5-14, the temperature of the air ranged from 14° to 19° C., and of the water, from 14.5° to 17° C.

TABLE XVIII.

DRAINAGE CANAL AT LOCKPORT.

Initial colony count sewage bacteria	- - - -	110,000
Initial colon bacilli	- - - - -	4,000-6,000
Initial Streptococci	- - - - -	1,000

B. typhosus, Strain (x), was added to this sewage in such numbers that each sac contained approximately 600 typhoid bacilli per cubic centimeter.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found	
1 day.....	C ₁	...	Drigalski	...	16	20	0
1 day.....		...	Horrocks	...	4		
2 days.....	C ₂	...	Drigalski	...	20	30	0
2 days.....		...	Horrocks	...	10		
3 days.....	"	1.0	Drigalski	230	30	50	0
3 days.....	"	1.0	Horrocks	130	10		
3 days.....	"	1.0	MacConkey	270	10	24	0
5 days.....	"	1.0	Drigalski	...	8		
5 days.....	"	1.0	Horrocks	...	8	12	0
5 days.....	"	1.0	MacConkey	...	8		
7 days.....	"	1.0	Drigalski	...	4	10	0
7 days.....	"	1.0	Horrocks	...	5		
9 days.....	"	1.0	Drigalski	...	4	10	0
9 days.....	"	1.0	Horrocks	...	1		
9 days.....	"	1.0	MacConkey	...	1		

During the conduct of this experiment, October 5-14, the temperature of the air ranged from 14° to 19° C., and of the water, from 14.5° to 17° C.

TABLE XIX.

DRAINAGE CANAL AT LOCKPORT.

Initial colony count sewage bacteria	- - - -	110,000
Initial colon bacilli	- - - - -	4,000-6,000
Initial streptococci	- - - - -	1,000

B. typhosus, Strain (y), was added to this sewage in such numbers that each sac contained approximately 180 typhoid bacilli per cubic centimeter. The bacilli were added in 5 c.c. of urine from a typhoid patient.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
1 day.....	D ₁	1.0	Drigalski	...	10	0
1 day.....	"	1.0	Horrocks	...	3	
3 days.....	"	1.0	Drigalski	...	5	0
3 days.....	"	1.0	Horrocks	...	8	
3 days.....	"	1.0	MacConkey	...	7	0
5 days.....	"	1.0	Drigalski	...	6	
5 days.....	"	1.0	MacConkey	...	6	0
7 days.....	"	1.0	Drigalski	...	8	
7 days.....	"	1.0	Horrocks	...	6	0
7 days.....	"	1.0	MacConkey	...	6	

During the conduct of this experiment, October 5-14, the temperature of the air ranged from 14° to 19° C., and of the water, from 14.5° to 17° C.

TABLE XX.

DRAINAGE CANAL AT LOCKPORT.

Initial colon bacilli	- - - - -	1,000
Initial streptococci	- - - - -	2,000

B. typhosus, Strain (y), was added to this sewage in such numbers that each sac contained approximately 360,000 typhoid bacilli per cubic centimeter.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
10 min.	O ₁	0.001	Drigalski	45	14	13 } 63
10 min.	"	0.001	Horrocks	160	18	
10 min.	"	0.001	Hiss	230	36	
2 days.....	"	0.1	Drigalski	410	15	0
2 days.....	"	0.1	Hiss	5,700	25	
2 days.....	"	0.01	Hiss	...	25	0
3 days.....	"	1.0	Drigalski	268	10	
3 days.....	"	0.1	Drigalski	...	10	0
3 days.....	"	1.0	MacConkey	270	10	
3 days.....	"	0.1	MacConkey	...	10	0
3 days.....	"	1.0	Horrocks	250	13	
3 days.....	"	0.1	Neutral agar	5,840	10	0
5 days.....	O ₂	0.1	Drigalski	...	3	
5 days.....	"	1.0	Drigalski	...	14	0
5 days.....	"	1.0	MacConkey	...	21	
5 days.....	"	0.1	Hiss	...	2	0
5 days.....	"	0.1	Neutral agar	...	6	
7 days.....	O ₁	1.0	MacConkey	126	8	0
7 days.....	"	1.0	Hiss	390	11	
7 days.....	O ₂	1.0	MacConkey	32	5	0
7 days.....	"	0.1	Hiss	370	18	
10 days.....	"	1.0	Hiss	85	85	0

During the conduct of this experiment, October 17-27, the temperature of the air ranged from 10° to 17.5° C., and of the water, from 10° to 14.5° C.

TABLE XXI.

DRAINAGE CANAL AT ROBEY STREET.

Initial colony count sewage bacteria 240,000
 Initial colon bacilli 1,000

B. typhosus, Strain (z), was added to this sewage in such numbers that each sac contained approximately 285,000 typhoid bacilli per cubic centimeter.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
1 hour.....	P ₁	0.001	Hiss	430	25	20
1 hour.....	"	0.001	Drigalski	80	6	4
1 hour.....	"	0.0001	Drigalski		3	2
1 hour.....	"	0.001	MacConkey	210	15	15
20 hours.....	"	0.001	Hiss	440	37	13
20 hours.....	"	0.001	Neutral agar	610	4	1
20 hours.....	"	0.001	Drigalski	15	6	2
20 hours.....	"	0.01	MacConkey	1,780	7	5
28 hours.....	"	0.01	Hiss	1,350	33	24
28 hours.....	"	0.001	Hiss		4	1
28 hours.....	"	0.01	Drigalski	60	3	0
28 hours.....	"	0.01	MacConkey	430	21	21
2 days.....	"	0.01	Hiss	173	59	1
2 days.....	"	0.01	MacConkey ¹		3	0
2 days.....	"	0.1	MacConkey	108	7	0
3 days.....	"	0.01	Hiss	182	25	31
3 days.....	"	0.1	Hiss		6	0
4 days.....	"	0.1	Hiss	3,400	15	21
4 days.....	"	1.0	Drigalski ²	45	6	0
5 days.....	"	0.1	Hiss	105	10	16
5 days.....	"	1.0	Drigalski	32	6	0
6 days.....	"	0.1	Hiss	1,344	32-32	0
7 days.....	"	0.1	Hiss	1,130	28	36
7 days.....	"	1.0	Drigalski	160	8	0
8 days.....	"	0.1	Hiss	440	31	35
8 days.....	"	1.0	MacConkey	120	4	0

During the conduct of this experiment, October 25 to November 4, the temperature of the air ranged from 6° to 13° C., and of the water, from 9.5° to 12° C.

TABLE XXII.

DRAINAGE CANAL AT ROBEY STREET.

Initial colony count sewage bacteria 240,000
 Initial colon bacilli 1,000

B. typhosus, Strain (z), was added to this sewage in such numbers that each sac contained approximately 285,000 typhoid bacilli per cubic centimeter.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
3 days.....	P ₂	0.01	Hiss	1,130	14	19
3 days.....	"	0.1	Drigalski ³	229	3	0
3 days.....	"	0.01	MacConkey	35	2	0
4 days.....	"	0.1	Hiss	6,120	13	18
4 days.....	"	1.0	MacConkey ⁴		5	0
5 days.....	"	0.1	Hiss	1,890	23	33
5 days.....	"	1.0	MacConkey	97	10	0
6 days.....	"	0.1	Hiss	1,540	15	23
6 days.....	"	1.0	Drigalski	83	8	0
7 days.....	"	0.1	Hiss	1,296	11	18
7 days.....	"	0.1	Neutral agar	670	5	0
7 days.....	"	1.0	Drigalski	44	2	0
8 days.....	"	0.1	Hiss	1,890	18-18	0
9 days.....	"	0.1	Hiss	1,960	8	0
9 days.....	"	0.1	Neutral agar	1,490	13	41
9 days.....	"	1.0	MacConkey	140	20	0
10 days.....	"	0.1	Hiss ⁵	1,820	24	0
10 days.....	"	1.0	MacConkey	86	13	3

During the conduct of this experiment, October 25 to November 4, the temperature of the air ranged from 6° to 13° C., and of the water, from 9.5° to 12° C.

¹ All typhoid-like colonies on MacConkey fished.

² All typhoid-like colonies on Drigalski fished.

³ All typhoid-like colonies on MacConkey and Drigalski fished.

⁴ All typhoid-like colonies on MacConkey fished.

⁵ All surface typhoid-like colonies on Hiss and MacConkey fished.

TABLE XXIII.

DRAINAGE CANAL AT ROBEY STREET.

Initial colony count sewage bacteria.....240,000
 Initial colon bacilli.....1,000

B. typhosus, Strain (y), was added to this sewage in such numbers that each sac contained approximately 857,000 typhoid bacilli per cubic centimeter. The bacilli were added in 50 c.c. of urine from a typhoid patient.

Time after Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
20 hours.....	N ₁	0.001	Hiss	730	17	5
20 hours.....	"	0.001	Neutral agar	1,040	2	0
20 hours.....	"	0.001	Drigalski	118	7	0
20 hours.....	"	0.01	Drigalski		15	6
20 hours.....	"	0.01	MacConkey	960	9	9
28 hours.....	"	0.01	Hiss	540	32	23
28 hours.....	"	0.01	Drigalski	380	15	10
28 hours.....	"	0.01	MacConkey	392	10	10
2 days.....	"	0.01	Hiss		23	3
2 days.....	"	0.1	Drigalski		14	0
2 days.....	"	0.01	MacConkey ¹		5	4
2 days.....	"	0.1	MacConkey		10	2
3 days.....	"	0.01	Hiss ²	1,072	36	
3 days.....	"	0.1	Drigalski	4,080	8	56
3 days.....	"	0.01	MacConkey	520	12	
4 days.....	"	0.01	Hiss ³	2,040	25	35
4 days.....	"	0.1	Hiss		10	
5 days.....	"	0.1	Hiss	7,200	16	23
5 days.....	"	1.0	Drigalski	230	7	
6 days.....	"	0.1	Neutral agar	1,490	4	10
6 days.....	"	1.0	Drigalski	180	6	
7 days.....	"	0.1	Hiss	1,890	25	25
8 days.....	"	0.1	Hiss	1,390	23	23
9 days.....	"	0.1	Hiss	17,400	13	
9 days.....	"	1.0	MacConkey	214	19	32

During the conduct of this experiment, October 25 to November 4, the temperature of the air ranged from 6° to 13° C., and of the water, from 9.5° to 12° C.

TABLE XXIV.

DRAINAGE CANAL AT ROBEY STREET—SUMMARY.

SAC No.	No. TYPH. BAC. INTROD.	1 HOUR		20 HOURS		28 HOURS		2 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
		c.c.		c.c.		c.c.		c.c.	
P ₁	285,000	0.0001... 3	2	0.001... 47	16	0.001... 4	1	0.01... 62	1
P ₂	285,000	0.001... 46	39	0.01... 7	5	0.01... 57	45	1.0... 7	0
N ₁	857,000		..	0.001... 26	5		..	0.01... 28	7
N ₂	857,000		..	0.01... 24	15	0.01... 57	43	0.1... 24	2
F ₁	11,500		..		..		..		..
F ₂	11,500		..		..		..		..
F ₃	11,500		..		..		..		..
G ₁	670		..		..		..	0.1... 25	0
G ₂	670		..		..		..	1.0... 10	0
H ₁	18,000		..		..		..		..
H ₂	18,000		..		..		..		..
H ₃	18,000		..		..		..		..
I ₁	900		..		..		..	0.1... 25	0
R ₁	25,000		..		..		..	1.0... 10	0
R ₂	25,000		..		..		..	0.1... 11	0
S ₁	16,800		..		..		..	1.0... 9	0
Total		49	41	104	41	118	89	221	10

¹ All typhoid-like colonies on MacConkey (0.01) fished.

² No surface typhoid-like colonies on Hiss (0.01) and Drigalski.

³ All surface typhoid-like colonies on Hiss fished.

TABLE XXIV.—Continued.

SAC No.	No. TYPH. BAC. IN- TROD.	3 DAYS		4 DAYS		5 DAYS		6 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
P ₁	285,000	c.c. 0.1.....25	0	c.c. 0.1.....15	0	c.c. 0.1.....10	0	c.c. 0.1.....32	0
		0.1.....6	0	1.0.....6	0	1.0.....6	0		
P ₂	285,000	0.01.....16	0	0.1.....13	0	0.1.....23	0		
		0.01.....3	0	1.0.....5	0	1.0.....10	0		
N ₁	857,000	0.01.....48	0	0.01.....25	0	0.1.....16	0	0.1.....4	0
		0.1.....8	0	0.1.....10	0	1.0.....7	0	1.0.....6	0
N ₂	857,000			0.01.....6	0	0.1.....15	0	1.0.....12	0
				0.1.....8	0	1.0.....14	0		
F ₁	11,500			1.0.....25	0			1.0.....20	0
F ₂	11,500								
F ₃	11,500								
G ₁	670			1.0.....15	0				
G ₂	670								
H ₁	18,000			1.0.....50	0			1.0.....19	0
H ₂	18,000								
H ₃	18,000								
I ₁	900			1.0.....15	0			1.0.....17	0
R ₁	25,000								
R ₂	25,000	0.001...19	0			0.01...12	0		
S ₁	16,800	0.0001...4	0			0.01...14	0		
		0.001...13	0						
Total		142	0	193	0	127	0	133	0

TABLE XXIV.—Continued.

SAC No.	No. TYPH. BAC. IN- TROD.	7 DAYS		8 DAYS		9 DAYS		10 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
P ₁	285,000	c.c. 0.1.....28	0	c.c. 0.1.....31	0	c.c.		c.c.	
		1.0.....8	0	1.0.....4	0				
P ₂	285,000	0.1.....16	0	0.1.....18	0	0.1.....21	0	0.1.....24	0
		1.0.....2	0			1.0.....20	0	1.0.....13	3
N ₁	857,000	1.0.....25	0	1.0.....23	0	0.1.....13	0		
						1.0.....19	0		
N ₂	857,000					0.1.....8	0	0.1.....35	0
						1.0.....21	0	1.0.....24	0
F ₁	11,500								
F ₂	11,500	1.0.....15	0						
F ₃	11,500								
G ₁	670								
G ₂	670	1.0.....23	0						
H ₁	18,000	1.0.....22	0						
H ₂	18,000								
H ₃	18,000								
I ₁	900								
R ₁	25,000								
R ₂	25,000								
S ₁	16,800								
Total		139	0	76	0	102	0	96	3

TABLE XXIV.—Continued.

SAC. No.	No. TYPH. BAC. IN- TROD.	11 DAYS		14 DAYS		12 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
	c.c.			c.c.		c.c.	
P ₁	285,000		..		..		..
P ₂	285,000		..		..		..
N ₁	857,000		..		..		..
N ₃	857,000		..		..		..
F ₁	11,500		..		..		..
F ₂	11,500	1.0.....19	0		..	1.0.....27	0
F ₃	11,500		..	1.0.....41	0		..
G ₁	670		..		..		..
G ₂	670		..		..		..
H ₁	18,000		..		..		..
H ₂	18,000	1.0.....14	0		..	1.0.....23	0
H ₃	18,000		..	1.0.....34	0		0
I ₁	900		..		..		..
R ₁	25,000		..		..		..
R ₂	25,000		..		..		..
S ₁	16,800		..		..		..
Total		33	0	75	0	50	0

Total colonies taken = 1,658

Total typhoid found = 184

Total colonies taken after 2 days = 1,166

Total typhoid found after 2 days = 3

TABLE XXV.

DRAINAGE CANAL AT LOCKPORT—SUMMARY.

Sac No.	No. Typh. Bac. Introd. per c.c.	10 MINUTES		1 DAY		2 DAYS		3 DAYS		5 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
	c.c.			c.c.		c.c.		c.c.		c.c.	
O ₁ ..	360,000	0.001....68	63		..	0.01....25	0	0.1....43	0		0
O ₂ ..	360,000		..		..	0.1....40	0	1.0....20	0	0.1.. 9	0
B ₁ ..	12,000		..	60	0		..		..	1.0..35	0
B ₂ ..	12,000		..		..		..	0.1.... 6	0	1.0..24	..
C ₁ ..	600		..	20	0	70	0	1.0....50	0	1.0..24	0
C ₂ ..	600		..		..		..	1.0....20	0	1.0..12	..
D ₁ ..	180		..	1.0....13	0	30	0	0.01.... 8	0		0
L ₁ ..	30,000		..		..		..	1.0....12	0		..
L ₂ ..	30,000		..		..		..	0.01.... 2	0	0.1.. 7	0
L ₃ ..	30,000		..		..		..	1.0....18	0	1.0.. 5	..
M ₁ ..	1,500		..		..		..	0.01.... 5	0	1.0..12	0
M ₂ ..	1,500		..		..		..	1.0.... 5	0	0.1.. 8	0
Total.....		68	63	93	0	165	0	202	0	136	0

TABLE XXV.—*Continued.*

Sac No.	No. Typh. Bac. Introd. per c.c.	6 DAYS		7 DAYS		8 DAYS		9 DAYS		10 DAYS	
		No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found	No. Col. Taken in	No. Typh. Found
O ₁ ..	360,000	c.c.	..	c.c.	..	c.c.	..	c.c.	..	c.c.	..
O ₂ ..	360,000		..	1.0.	19 0		..		..		..
			..	0.1.	18 0		..		..	1.0.	85 0
			..	1.0.	5 0		..		..		..
B ₁ ..	12,000		..		..		..		..		..
B ₂ ..	12,000		..	1.0.	45 0		..	1.0.	49 0		..
C ₁ ..	600		..		..		..		..		..
C ₂ ..	600		..	1.0.	12 0		..	1.0.	10 0		..
D ₁ ..	480		..	1.0.	20 0		..		..		..
L ₁ ..	30,000	1.0.	17 0		..	1.0.	24 0		..	1.0.	26 0
L ₂ ..	30,000		..		..		..		..		..
L ₃ ..	30,000*		..		..		..		..		..
M ₁ ..	1,500		..		..		..		..		..
M ₂ ..	1,500		..		..		..		..		..
Total.....		17	0	119	0	24	0	59	0	111	0

Total colonies taken = 992

Total typhoid found = 63

Total colonies taken after day of inoculation = 924

Total typhoid found after 10 minutes = 0

The tabular résumé shows that, with one exception, no typhoid bacilli were found in plates made from any of the sacs later than two days after inoculation. The decrease in numbers seems to be most marked between twenty-four and forty-eight hours. In most instances all the typhoid-like colonies on the plate were taken, and although 1,166 colonies in all were examined, only three of these proved to be typhoid. In one case all the colonies on the plate (1 c.c.) were picked off and examined (Table XX, 10 days).

Regarding the finding of three colonies of typhoid bacilli on the tenth day after inoculation (Table XXII), it should be noted that these three colonies all appeared on a single plate, and although a number of plates from this same sac had been made during the preceding six days, no typhoid colonies whatever had been discovered. This sudden appearance of typhoid bacilli on a single plate creates the suspicion that their survival may have been due to some accidental cause, and that they had not persisted in the water of the sac during the whole period of the experiment. It seems a plausible supposition that these bacilli

may have adhered to some concrete particle in the sewage when they were freshly introduced, and that this particle dried upon the walls of the tube and remained there until accidentally washed off during the process of agitating the contents of the sac. It is, of course, possible that these three bacilli were unusually resistant individual cells which maintained their vitality in the water of the sac throughout the period covered by the experiment. This, however, seems unlikely, in view of all the other findings in the course of this investigation, and the circumstances under which these bacilli appeared indicate that their occurrence was an accidental instead of a significant one.

In addition to the sacs of sewage infected with typhoid bacilli, a number of control experiments were carried out upon uninoculated sewage (Table XXVI). The number of colonies developing on neutral agar plates, and also the number of colon bacilli and streptococci, were determined. The results obtained in this way afford an interesting confirmation of the results obtained with the typhoid-infected sacs. The number of colon bacilli remains nearly constant for about twenty-four hours, and then rapidly diminishes, falling, after five or six days, to a point far below the initial number. The streptococci, while sometimes more numerous at the outset than the colon bacilli, fall more rapidly and practically disappear within a few days after the opening of the experiment. The number of the colonies developing on agar plates shows a slight increase in the first twenty-four hours, and then a decline which is noticeably rapid and extreme.

TABLE XXVI.
CONTROLS (UNINOCULATED SEWAGE).
LOCKPORT.

No. Days in Sac	Sac No.	Colony Count Agar 37°	No. B. coli in 1 c.c.	No. Streptococci in 1 c.c.
Initial	A ₁	470,000	4,000-6,000	1,000
3 days	A ₂	2,000	Less than 1,000	1,000
7 days	A ₂	660	50	None in 1 c.c.
9 days	A ₃	2,520	5	None in 10 c.c.
Initial	K ₁	?	1,000	2,000
3 days	K ₁	8,000	10	20
5 days	K ₂		6-9	None in 5 c.c.
6 days	K ₂	1,332	6-8	None in 5 c.c.
8 days	K ₁	11,800	7	

ROBEY STREET.

No. Days in Sac	Sac No.	Colony Count Agar 37°	No. B. coli in 1 c.c.	No. Streptococci in 1 c.c.
Initial.....	E ₁	390,000	4,000-6,000	40,000
2 days	E ₄	21,000	100	20
4 days	E ₁	19,500	70-90	1 in 5 c.c.
6 days	E ₁	70,200	50	None in ½ c.c.
7 days	E ₂	17,200	90-100	None in 1-10 c.c.
11 days	E ₂	14,600	20	None in 1-5 c.c.
12 days	E ₃	75,600	4-10	None in 2-5 c.c.
14 days	E ₃	Too thick to count	6-10	
Initial.....	V ₁	240,000	800-1,000	
20 hours	V ₁	327,000	700-900	
28 hours	V ₁	326,000	800	
2 days	V ₁	122,000	100-200	
4 days	V ₁	17,000	20	
5 days	V ₁	16,400	7-8	
6 days	V ₁	17,800	5-6	
7 days	V ₁	7,900	8	
8 days	V ₁	5,980	9	
9 days	V ₁	7,300	6-8	

It may be concluded, therefore, that the vast majority of the typhoid bacilli introduced into the sewage of the Chicago Drainage Canal, under the conditions which prevailed during the conduct of this experiment, disappear within two days after their introduction, and that while it may be true that individual cells endowed with special powers of resistance maintain their vitality for a longer period, the outcome of this experiment shows that such an assumption finds little warrant. Furthermore, assuming that such survival of individual typhoid bacilli with rare powers of resistance did sometimes occur, there is some ground for supposing that such adaptation to a saprophytic mode of life might be associated with a lowered virulence. The facts instanced by Hankin,¹ and by Remlinger and Schneider,² regarding the occasional presence in natural waters of typhoid bacilli apparently possessed of slight virulence, or even wholly innocuous, are sufficient to demand careful consideration of this possibility.

One experiment was also made upon Lake Michigan (tap) water for the purpose of comparing the course of events in the parchment and celloidin sacs under similar conditions. Large celloidin sacs with a capacity of about 700 c.c. were prepared, and these were suspended in running water at 16°C. side by side with parchment sacs. The experiment shows that even when a small number of typhoid bacilli (3,080) were introduced into the lake

¹ *Centralbl. f. Bakt.*, 1899, 26, p. 504.² *Ann. de l'Inst. Past.*, 1897, 11, p. 55.

water, survivors could be found four days after inoculation, or twice as long as in the water of the Drainage Canal. Incidentally the experiment affords confirmatory evidence of the validity of the methods of isolation employed, since the presence of very large numbers of water bacteria (over 100,000) did not prevent the discovery of the typhoid bacilli. (Cf. Table XXVII, 1, 2, and 3 days.)

TABLE XXVII.

B. typhosus, Strain (y), was added to tap water, colony count 65, in such numbers that each sac contained approximately 3,080 typhoid bacilli per cubic centimeter.

PARCHMENT SAC						CELLOIDIN SAC					
Time After In-oculation	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colo-nies Found	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colo-nies Found	
1 day	0.1	Hiss	2,800	12 } 21	10 } 19	0.1	Hiss	116,000	11 } 16	3 } 6	
1 day	1.0	MacConkey	1,600	9 } 10	9 } 9	1.0	MacConkey	1,860	5 } 12	3 } 2	
2 days	0.1	Hiss	31,200	10 } 20	2 } 9	0.1	Hiss	88,000	12 } 20	1 } 2	
2 days	1.0	MacConkey	1,550	10 } 14	7 } 5	1.0	MacConkey	2,400	8 } 20	1 } 0	
3 days	0.1	Hiss	121,400	14 } 20	0 } 5	0.1	Hiss	110,400	14 } 20	0 } 0	
3 days	1.0	MacConkey	2,810	6 } 14	5 } 1	1.0	MacConkey	9,840	6 } 20	0 } 1	
4 days	0.1	Hiss	119,200	14 } 20	1 } 1	0.1	Hiss	36,800	14 } 20	1 } 1	
4 days	1.0	MacConkey	2,720	6 } 16	0 } 0	1.0	MacConkey	4,188	6 } 20	0 } 0	
5 days	0.1	Hiss	118,000	16 } 20	0 } 0	0.1	Hiss	20,100	16 } 20	0 } 0	
5 days	1.0	MacConkey	2,160	4 } 16	0 } 0	1.0	MacConkey	1,900	4 } 20	0 } 0	
6 days	0.1	Hiss	31,200	16 } 20	0 } 0	0.1	Hiss	10,440	16 } 20	0 } 0	
6 days	1.0	MacConkey	2,600	4 } 14	0 } 0	1.0	MacConkey	1,060	4 } 20	0 } 0	
7 days	0.1	Hiss	29,800	14 } 17	0 } 0	0.1	Hiss	9,200	12 } 17	0 } 0	
7 days	1.0	MacConkey	2,520	3 } 16	0 } 0	1.0	MacConkey	830	5 } 16	0 } 0	
8 days	1.0	Hiss	26,800	16 } 20	0 } 0	1.0	Hiss	3,480	16 } 20	0 } 0	
8 days	1.0	MacConkey	1,670	4 } 20	0 } 0	1.0	MacConkey	480	4 } 18	0 } 0	
9 days	1.0	Hiss	6,240	16 } 20	0 } 0	1.0	Hiss	3,160	18 } 22	0 } 0	
9 days	1.0	Drigalski	1,720	4 } 16	0 } 0	1.0	Drigalski	980	4 } 13	0 } 0	
11 days	1.0	Hiss	3,400	16 } 20	0 } 0	1.0	Hiss	520	13 } 20	0 } 0	
11 days	1.0	Drigalski	720	4 } 16	0 } 0	1.0	Drigalski	72	7 } 16	0 } 0	
12 days	1.0	Hiss	5,040	16 } 20	0 } 0	1.0	Hiss	410	16 } 20	0 } 0	
12 days	1.0	MacConkey	740	4 } 12	0 } 0	1.0	MacConkey	51	4 } 20	0 } 0	
13 days	1.0	Hiss	7,100	12 } 20	0 } 0	1.0	Hiss	2,400	0 } 20	0 } 0	
13 days	1.0	MacConkey	967	8 } 12	0 } 0	1.0	MacConkey	420	20 } 4	0 } 0	
15 days	1.0	Hiss	5,640	12 } 20	0 } 0	1.0	Hiss	550	4 } 20	0 } 0	
15 days	1.0	MacConkey	926	8 } 20	0 } 0	1.0	MacConkey	180	16 } 20	0 } 0	

LONGEVITY OF PARATYPHOID BACILLI.

In addition to the experiments that were carried out with the typhoid bacillus, one test was made of the longevity of the paratyphoid bacillus (Paratyphoid Scott) in the water of the Drainage Canal at Robey Street. The culture used was one isolated in this laboratory in June, 1903, about three months before these experiments were undertaken.¹

The methods used in the conduct of this experiment were precisely similar to those that have been described in the work upon *B. typhosus*, and the method of tabulation of the result is also similar (Tables XXVIII and XXIX). In the case of this organism a general correspondence was observed with the behavior of the typhoid bacillus, the most rapid disappearance

TABLE XXVIII.

DRAINAGE CANAL AT ROBEY STREET.

Initial colony count sewage bacteria - - - - - 240,000
Initial colon bacilli - - - - - 1,000

B. paratyphosus, Strain (pt), was added to this sewage in such numbers that each sac contained approximately 820,000 paratyphoid bacilli per cubic centimeter.

Time After Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Typhoid Colonies Found
20 hours.....	T ₁	0.01	Hiss	4,840	40	22
20 hours.....	"	0.001	Neutral agar	550	3	1
20 hours.....	"	0.001	Drigalski	61	8	4
20 hours.....	"	0.01	Drigalski		9	2
20 hours.....	"	0.01	MacConkey	230	3	1
28 hours.....	"	0.001	Hiss ²	44	6	2
28 hours.....	"	0.01	Hiss		43	20
28 hours.....	"	0.01	Drigalski	610	4	4
28 hours.....	"	0.01	MacConkey	230	2	0
2 days.....	"	0.01	Hiss		35	2
2 days.....	"	0.01	Drigalski ³		6	1
2 days.....	"	0.1	MacConkey		8	0
3 days.....	"	0.01	Hiss	300	16	0
3 days.....	"	0.1	Hiss		7	0
3 days.....	"	0.1	Drigalski	107	10	2
4 days.....	"	0.1	Hiss	3,480	15	15
5 days.....	"	0.1	Hiss	2,720	16	16
5 days.....	"	0.1	Neutral agar	456	8	31
5 days.....	"	1.0	MacConkey	37	7	7
6 days.....	"	0.1	Hiss	1,688	13	19
6 days.....	"	1.0	Drigalski	280	6	6
7 days.....	"	0.1	Hiss	1,410	15	25
7 days.....	"	0.1	Neutral agar	1,079	10	10
8 days.....	"	0.1	Hiss	1,310	14	17
8 days.....	"	1.0	MacConkey	14	3	3

During the conduct of this experiment, October 25 to November 4, the temperature of the air ranged from 6° to 13° C., and of the water, from 9.5° to 12° C.

¹ WELLS AND SCOTT, *Jour. Infect. Dis.*, 1904, 1, p. 72.

² All typhoid-like colonies on Drigalski, MacConkey, and Hiss (0.001) fished.

³ All typhoid-like colonies on Drigalski (0.01) and MacConkey (0.1) fished.

appearing to take place within the first forty-eight hours after inoculation. Two colonies were found on the third day after inoculation, but thereafter up to the eighth day, in two sets of sacs, no paratyphoid colonies were discovered. These experiments appear to indicate that the life of this organism in the water of the Drainage Canal, although possibly slightly more extended than that of the typhoid bacillus, is still very brief.

TABLE XXIX.

DRAINAGE CANAL AT ROBEY STREET.

Initial colony count sewage bacteria - - - - - 240,000

Initial colon bacilli - - - - - 1,000

B. paratyphosus, Strain (pt), was added to this sewage in such numbers that each sac contained approximately 820,000 paratyphoid bacilli per cubic centimeter.

Time After Inoculation	Sac No.	Amount Plated in c.c.	Medium	Colonies on Plate	Colonies Fished	Paratyph. Colonies Found
4 days	T ₂	0.1	Hiss	7,380	19 } 25	0
4 days	"	1.0	MacConkey	240	6 }	
5 days	"	0.1	Hiss	3,040	16 }	
5 days	"	0.1	Neutral agar	2,480	2 } 24	0
5 days	"	1.0	MacConkey	83	6 }	
6 days	"	0.1	Hiss	2,200	6 } 6	0
8 days	"	0.1	Neutral agar	860	6 } 6	0

During the conduct of this experiment, October 25 to November 4, the temperature of the air ranged from 6° to 13° C., and of the water, from 9.5° to 12° C.

EXPERIMENTS CONDUCTED IN THE ILLINOIS RIVER AT AVERYVILLE, ILLINOIS (H. L. R.).

Before giving the results of the experiment made at this station, the following observations, collected on the temperature conditions of the river and the stage of water, are presented:

TABLE XXX.

DATA AS TO CONDITIONS IN RIVER (STAGE OF WATER, TEMPERATURE, AND TURBIDITY).

Date	Temperature of Water (°C)	Temperature of Air (°C—9 A. M.)	Stage (Feet above Low-Water Mark)
October 10	17.0	11.0	11.7
October 12	16.5	14.0	11.4
October 13	16.0	17.0	11.4
October 14	16.0	15.0	11.4
October 15	16.0	15.5	11.3
October 16	16.0	15.0	11.3
October 19	14.0	11.0	11.1
October 20			11.0
October 21	14.0	16.0	11.0
October 23	13.0	8.0	11.0
October 26	11.0	6.0	10.8

As the matter of temperature is of material importance in affecting the vitality of organisms, it should be kept in mind that the temperature to which the typhoid organisms were exposed in this case was materially below the mean summer heat, and would therefore favor the retention of vitality to some extent, as far as this factor is operative. Regarding the stage of the river, the data show very uniform conditions. The lowest stage noted during this summer was about eight feet above low water. At the time these experiments were conducted the turbidity in the stream was somewhat higher than usual, objects not being visible at the depth of a foot or more.

Three series of tests were made at Averyville, using fresh cultures of the three strains of the typhoid organism isolated by Professor Jordan, and designated by him as "x," "y," and "z."

In the "x" series five parchment sacs were infected; in the "y" series, an equal number; and in the "z" series, four, making in all fourteen different exposures. The "x" and "y" series were placed in the river on October 9, 1903, and "z" cultures on October 14. Samples were removed from some of the sacs at daily intervals until the end of the experiment (October 26). The first two series were therefore exposed to the influence of the river water for a period of seventeen days, and the "z" series for twelve days.

In addition to these sacs, which were first inoculated with river water and then infected with varying amounts of typhoid cultures, two other sacs filled with raw water, but uninfected with typhoid, were kept under the same conditions as controls. These were examined at intervals in order to learn the course of the bacterial changes which went on in the parchment sacs when filled with the raw water alone.

It is a well-known fact that when a small quantity of water is inclosed in a glass container, a rapid and extensive multiplication of water bacteria occurs. Just what forces are responsible for this marked proliferation is not fully understood, but the variation in experimental conditions which obtained in these experiments as to character of container rendered it especially desirable that data should be secured on this point.

Samples were therefore removed at intervals and plated on Drigalski and Conradi's medium. The results of these tests showed an increase in number of organisms per cubic centimeter, but this was only a few fold and maintained for a brief period of time. It was impossible, for lack of time, to make a study of the quantitative changes on plain agar, but doubtless it would have been greater than here reported. The results for one of these control sacs are tabulated below:

TABLE XXXI.
BACTERIAL CONTENT PER CUBIC CENTIMETER OF PARCHMENT SACS FILLED
WITH RAW WATER AND IMMersed IN RIVER.

NO. DAYS' IMMERSION IN RIVER WATER	PERIOD OF INCUBATION OF CULTURES			
	1 Day	2 Days	3 Days	4 Days
1	6,000			32,000
2	18,000		68,000	
5	2,700	5,500		
7	20			
9	90			

From these data it appears that the use of the selective typhoid medium (Drigalski and Conradi) did not succeed in inhibiting entirely the growth of some of the water forms, although these indigenous species rapidly disappeared when cultivated in this medium. Attempts were made in the typhoid-infected sacs to obtain some general idea of the rate of bacterial changes. Of course, here the problem was complicated by the presence of the typhoid forms which are not greatly affected when cultivated in this culture medium. The general course of these changes was quite similar in all cases. The maximum germ content in such cases on Drigalski and Conradi's medium was found at first or within a couple of days. After this a rapid decline set in, so that, when the sacs were withdrawn from the river at the expiration of the work, they contained only a very few bacteria capable of growing on this medium.

Ratio of typhoid to indigenous water organisms.—Reference has been previously made to the question of seeding, as to the importance of knowing approximately how many typhoid organ-

isms were exposed to the water, and the effect which increased seedings have on the vitality of organisms. Under the conditions of exposure in the river, the ratio of typhoid organisms to the bacteria normally present in the river water was very much greater than could possibly occur in nature. The extremes have been previously given, but the estimated seedings for the different sacs are herewith presented in detail:

TABLE XXXII.
VARIATION IN TYPHOID SEEDINGS IN DIFFERENT SACS.

Series "x"		Series "y"		Series "z"	
Sac 1.....	1,000	Sac 6.....	1,000	Sac 12.....	540
2.....	2,000	7.....	2,000	13.....	540
3.....	4,000	8.....	4,000	14.....	5,400
4.....	10,000	9.....	10,000	15.....	10,800
5.....	20,000	10.....	20,000		

When it is remembered that the normal germ content on plain agar at blood heat of the river water at this station ranges from 60 to 500, it is at once apparent that the extent of typhoid infection is here vastly greater than would normally occur under natural conditions.

As a matter of fact, the culture plates made from the infected sacs at this station rarely showed the presence of acid colonies. When the first series of cultures were prepared, a large number of colonies developed, but these fell off rapidly. This fact greatly simplified the labor of selecting the presumptive typhoid colonies from the plates. In the later stages the colonies became more atypical for typhoid and much reduced in numbers. Under these conditions relatively large numbers were taken, so as to insure finding typhoid if present.

In making cultures from the water, sets of at least three plates were prepared with Drigalski & Conradi's medium, using a somewhat wide range in dilution (1:20 to 1:2,000 c.c.). This permitted the selection of plates having a satisfactory distribution of colonies. As the germ content declined, the amount of liquid used in seeding was increased, until, toward the end, plates were prepared containing 1 c.c. of water. Ordinarily the plates were studied the first day, the supposedly typhoid colonies being fished

and inoculated into glucose agar, then incubated. The plates were further incubated at 20° C. for another twenty-four hours and gone over again. The sugar agar cultures were then inspected, all showing gas being thrown out, and the balance sent to Madison for further study. The culture methods followed here were essentially the same as used by E. O. J. at Chicago, viz.: litmus milk, dextrose litmus broth, glucose-free broth for indol, and nutrient gelatin. Cultures that showed positive typhoid characters on these culture media were then subjected to the agglutination test, using a homologous serum of 1:500. They were also subjected to a highly potent typhoid serum secured from Parke, Davis & Co. In the subjoined tables are given the results of the various examinations, including, as far as possible, the data as to total number of colonies fished and number found to be typhoid. As the entire work of plate-culturing at Peoria devolved upon Mr. Hastings, it was impossible for him at times to compute the total colony count on each set examined, but the data collected are sufficient to show a steady and often quite rapid decline in total number of appearing colonies.

In testing these various sacs emphasis was placed in each series on the minimum and maximum seeding. If the typhoid organism could be demonstrated in these two extremes, it was, of course, highly probable that intermediate seedings would fall within these limits. In the "x" series positive results were not secured with the frequency noted in the remaining series. This was due to the fact that the parchment tubing was soaked in river water before being seeded with the typhoid culture. Under these conditions it became somewhat fouled with suspended sediment, thereby much increasing the germ content of the inclosed water for a time.

It is evident from the data submitted in Tables XXXIII-XXXV that the amount of seeding was sufficient to permit of the detection of the organism, if it was present at all. The sacs containing one-twentieth as many typhoid organisms as the sac seeded with the maximum quantity showed in both the "y" and "z" series the presence of the organism for as long a period as in any case.

It was unfortunate that a larger number of the sacs were not

tested until after the lapse of several days, but we had no expectation that the organisms introduced would die off so rapidly. It should be kept in mind that the final records as to whether the isolated cultures were true typhoid or not were not made until long after the removal of the sacs from the water. Therefore it was not possible to check up the earlier gaps in the analytical records. During these earlier days the typhoid organisms were doubtless more or less abundant, but the much larger number of tests made on the greatly reduced germ content of the sacs after the first few days, and the practical absence of the introduced organism, greatly strengthened the conclusion that the typhoid germ had lost its vitality in a relatively short period of time.

The detailed data that were collected from the examination of the various sacs of the three series are presented in the following tables:

TABLE XXXIII.

RESULTS OBTAINED FROM CULTURES MADE FROM PARCHMENT SACS AFTER VARYING PERIODS OF EXPOSURE IN RIVER WATER.

SERIES INFECTED WITH "X" CULTURE.

No. of Sacs	Dosage of Typh. Bacilli per c.c.	Time after Inoculation When Examined—Days	Total No. of Colonies on Plates Made	No. of Colonies Fished	No. of Typhoid Colonies
1.....	1,000	1	330	12	1
		3	300	9	0
		5	25	11	0
		10	125	10	0
2.....	2,000	4	100	8	0
3.....	4,000	6	...	12	0
4.....	10,000	4	50	13	0
		5	...	3	0
		7	37	8	0
5.....	20,000	1	75	6	0
		3	25	13	2
		5	12	9	0
		6	...	8	0
		7	200	7	0
		10	100	12	0
		12	540	12	0
		14	288	11	0
		17	135	15	0

The results in this first series were not as satisfactory as in the remaining series, because of the fouling of the sacs with mud before inoculation. The sacs were placed in the river water to soak up before they were infected, and some sediment from river

water settled on them. The results obtained in these series showed the presence of the typhoid organism in sacs containing the minimum and maximum amount of typhoid seeding. In the latter case, containing 20,000 typhoid bacilli per cubic centimeter, nine different examinations were made, ninety-three colonies in all being fished from the culture plates. The inoculated organism was not found subsequent to the third day. Of this number seventy-four colonies were examined after the third day, and in no case was any organism found which resembled the typhoid germ in culture characters or in agglutinating tests.

TABLE XXXIV.

RESULTS OBTAINED FROM CULTURES MADE FROM PARCHMENT SACS AFTER VARYING PERIODS OF EXPOSURE IN RIVER WATER.
SERIES INFECTED WITH "Y" CULTURE.

No. of Sacs	Approximate Dosage of Typh. Bacilli per c.c.	Time after Inoculation When Examined—Days	Total No. of Colonies on Plates Made	No. of Colonies Fished	No. of Typhoid Colonies
6.....	1,000	1	19	8	3
		3	25	6	2
		14	68	9	0
		17	6		
7.....	2,000	4	3,600	9	0
		12	28	5	0
		14	185	5	0
		17	15	6	0
8.....	4,000	6		8	0
		10	73	6	0
		12	35	4	0
		17	47	8	0
9.....	10,000	4	11,500	11	0
		7	43	7	0
		10	160	10	0
		12	107	5	0
10.....	20,000	1	158	8	4
		3	100	16	10
		5		18	0
		7	103	6	0
		10	190	7	0

The "y" series was placed in the river at the same time as "x." Practically the same results were secured. In all the sacs examined up to the third day after immersion, the typhoid organism was detected where both light and heavy seeding was used. In none of the examinations after the third day (sixteen in number) was any evidence found which would lead to the belief that this specific germ had retained its vitality in any of the sacs for a longer period than three days.

TABLE XXXV.

RESULTS OBTAINED FROM CULTURES MADE FROM PARCHMENT SACS AFTER
VARYING PERIODS OF EXPOSURE IN RIVER WATER.

SERIES INFECTED WITH "Z" CULTURE.

No. of Sacs	Dosage of Typh. Bacilli per c.c.	Time after Inoculation When Examined—Days	Total No. of Colonies on Plates Made	No. of Colonies Fished	No. of Typhoid Colonies
12.....	540	1/24	50	3	3
		1	360	17	15
		2	350	12	6
		5	100	8	0
		12	4	2	0
13.....	540	7	7	3	0
		9		4	0
		12		7	0
14.....	5,400	1/24	53	3	3
		7	6	1	0
15.....	10,800	1	1,500	15	14
		2	4,000	13	6
		5	750	11	0
		10	430	12	1
		12	3	2	0

In this third series, in which the "z" culture isolated from feces was used, the seedings were in each case only about one-half of what was employed in the other tests. This test was not continued for as long a period as the other two, but the specific organism was recovered easily from all tubes examined shortly after inoculation, also on the first and on the second day. No tests happened to be made between the second and the fifth day, but on the tests made on this latter date and subsequently the typhoid organism was not found in any case, with one single exception. On the ninth day, among twelve colonies removed from sac No. 15, containing the maximum amount of typhoid seeding, one was found which responded to all the cultural tests for typhoid as well as the agglutination test in dilutions of 1 : 1,000. Examinations made from this same sac on the twelfth day showed no similar colonies. It is impossible to explain the presence of this single colony, appearing by itself eight or nine days later than any other typhoid colonies in this or any other sac. It is well known, of course, that the vitality of all individual organisms of the same species is not absolutely the same, but it seems highly improbable that the appearance of this single germ,

among the hundreds of colonies examined subsequent to the third day, can be attributed to a protracted retention of vitality much beyond the usual period.

A general summary of the foregoing detailed results, as presented in the appended tables, may perhaps aid in the interpretation of the analyses, if they are grouped according to the time of exposure. In Table XXXVI is shown the typhoid findings in the different sacs on the different days in which they were examined.

TABLE XXXVI.

NUMBER OF TYPHOID ORGANISMS RECOVERED FROM DIFFERENT SACS ON DIFFERENT DAYS.

Series	Sac No.	1D	2D	3D	4D	5D	6D	7D	8D	9D	10D	11D	12D	13D	14D	15D	16D	17D
X.....	1.....	1	..	0	..	0	..	..	..	..	0	..	..	..	..	..	..	..
	2.....	..	..	..	0	..	..	..	..	..	..	..	..	..	..	..	..	..
	3.....	..	..	..	..	0	..	..	..	..	..	..	..	..	..	..	..	..
	4.....	..	..	..	0	0	..	0	..	..	..	..	..	..	..	..	..	..
	5.....	0	2	..	..	0	0	0	..	..	0	..	0	..	0	..	..	0
Y.....	6.....	3	2	..	..	..	..	..	..	..	..	..	..	0	..	..	..	..
	7.....	..	0	0	..	..	..	..	..	..	..	0	..	0	..	0	..	..
	8.....	..	..	..	..	..	0	..	..	..	0	..	0	..	..	0	..	..
	9.....	..	..	..	0	..	..	0	..	..	0	..	0	..	..	..	..	..
	10.....	4	..	10	..	0	..	0	..	..	0	..	..	..	..	..	..	..
Z.....	12.....	3 ¹ 15	6	..	..	0	..	..	..	..	..	0	..	..	..	..	..	..
	13.....	..	..	..	..	..	0	..	0	..	..	0	..	..	..	..	..	..
	14.....	3 ¹	..	..	..	..	0	..	..	..	..	..	0	..	..	..	..	..
	15.....	14	6	..	..	0	..	..	..	1	..	..	0	..	..	..	..	..

Table XXXVII gives the total number of colonies from all sacs that were tested from day to day with the typhoid findings; also the distribution of the typhoid findings among the respective sacs.

TABLE XXXVII.

GENERAL SUMMARY OF RESULTS OBTAINED FROM EXAMINATIONS OF TYPHOID-INFECTED SACS MADE ON SUCCESSIVE DAYS.

	1	2	3	4	5	6	7	9	10	13	14	16	17	Totals
Number colonies tested by sub-cultures from all sacs.....	72	25	44	41	61	28	32	18	46	38	25	14	15	459
Number typhoid colonies found in various sacs on successive days.....	43	12	14	0	0	0	0	1	0	0	0	0	0	70
Total number times all sacs were examined.....	8	2	5	4	6	3	6	2	5	7	3	2	1	54
Total number times in which typhoid colonies were found...	7	2	3	0	0	0	0	1	0	0	0	0	0	13

¹ Indicates cases where examination was made one hour after examination.

From the foregoing tables it appears that there is a striking uniformity in the results which have been obtained in all three series made with different strains of the typhoid organism derived from entirely different sources. In each case the inoculated organism persisted in the sacs containing the minimum and maximum amount of seeding for the same period of time.

Vitality was retained as a rule in the waters of the Illinois river no longer than an interval of three days after infection. In only a single instance was any typhoid germ isolated after this period, and that case was on the ninth day. A rational explanation for the presence of this single colony is not apparent, but, in the judgment of the writer (H. L. R.) the presence of this particular organism is not believed to have any special significance in the problem under consideration.

GENERAL CONCLUSIONS.

1. From the experiments recorded in this paper it appears that under conditions that probably closely simulate those in nature the vast majority of typhoid bacilli introduced into the several waters studied perished within three to four days.

2. It is theoretically possible that specially resistant cells may occur which are able to withstand for a longer period the hostile influences evidently present in water. Our experiments, however, show that if such resistant individuals exist they must be very few in number and constitute only a small fraction of the bacilli originally entering the water.

3. It is not the intention of the writers to claim that the behavior of typhoid bacilli under the conditions herein described is representative of all conditions obtaining in all natural bodies of water.